



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| <b>(21) International Application Number:</b> PCT/US89/01088<br><b>(22) International Filing Date:</b> 16 March 1989 (16.03.89)<br><b>(31) Priority Application Numbers:</b> 169,618<br>323,350<br><b>(32) Priority Dates:</b> 16 March 1988 (16.03.88)<br>14 March 1989 (14.03.89)<br><b>(33) Priority Country:</b> US<br><br><b>(71) Applicant:</b> SCRIPPS CLINIC AND RESEARCH<br>FOUNDATION [US/US]; 10666 North Torrey Pines<br>Road, La Jolla, CA 92037 (US).<br><b>(72) Inventors:</b> CARSON, Dennis, A. ; 14824 Vista del Oc-<br>eano, Del Mar, CA 92014 (US). CARRERA, Carlos,<br>J. ; 3924, Canning Avenue, San Diego, CA 92111<br>(US). |           | <b>(74) Agents:</b> GAMSON, Edward, P. ; Dressler, Goldsmith,<br>Shore, Sutker & Milnamow, Ltd., 1800 Prudential<br>Plaza, Chicago, IL 60601 (US) et al.<br><br><b>(81) Designated States:</b> AT (European patent), AU, BE (Eu-<br>ropean patent), CH (European patent), DE (Euro-<br>pean patent), DK, FI, FR (European patent), GB<br>(European patent), IT (European patent), JP, KR, LU<br>(European patent), NL (European patent), NO, SE<br>(European patent).<br><br><b>Published</b><br><i>With international search report.</i><br><i>Before the expiration of the time limit for amending the</i><br><i>claims and to be republished in the event of the receipt</i><br><i>of amendments.</i> |
| <b>(54) Title:</b> SUBSTITUTED ADENINE DERIVATIVES USEFUL AS THERAPEUTIC AGENTS<br><br><b>(57) Abstract</b><br><br>Novel adenine derivatives whose structures are represented by formula (I), are disclosed, as are methods of using those compounds and others of formula (II) to treat monocyte-mediated disorders and autoimmune diseases.   |           |  |

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SUBSTITUTED ADENINE DERIVATIVES USEFUL  
AS THERAPEUTIC AGENTS

Description

5 This application is a continuation-in-part of  
co-pending Serial No. 169,618, filed March 16, 1988, whose  
disclosure is incorporated herein by reference.

Technical Field

10 This invention relates to agents that are useful  
in the treatment of chronic inflammatory diseases,  
infection, and autoimmune disorders. More particularly,  
this invention relates to a compound and a method of  
treatment for monocyte-mediated diseases or disease states  
(disorders). In one specific aspect, this invention  
pertains to a method for treating diseases in which a  
15 pathogen resides in monocytes. In a second specific  
aspect, this invention pertains to the treatment of  
autoimmune disorders, or other chronic diseases in which  
monocyte activation contributes to the pathology of the  
disease.

20 Background of the Invention

Recent reports indicate that human immune  
deficiency virus (HIV) infects macrophages and monocytes  
in addition to T cells. Levy et al. (1985) Virology,  
147:441-448; Gartner et al. (1986) Science, 233:215-219;  
25 and Wiley et al. (1986) Proc. Natl. Acad. Sci. USA,  
83:7089-7093. Acquired immune deficiency syndrome (AIDS)  
results from infection with HIV, also known as human  
T-lymphotropic virus Type III (HTLV-III).

30 AIDS is characterized by extensive  
immunosuppression that predisposes patients to life-  
threatening opportunistic infections as well as unusual  
forms of neoplasm. As to the other known subgroups or  
types of human T-lymphotropic viruses, Type I (HTLV-I) is  
believed to cause T cell proliferation in leukemia. The

role of HTLV-II in pathogenesis remains unclear, although it has been associated with rare cases of the T cell variant of hairy cell leukemia. Golde et al. (1986), *Seminars in Hematol.* 23:3-9.

5               Synthesis of DNA complementary to viral RNA is thought to be required for both retroviral integration into host DNA and for the generation of new virions. For this reason, the HIV-encoded reverse transcriptase is a logical target for the development of agents for the  
10              treatment of patients with the acquired immunodeficiency syndrome [De Clercq et al. (1986) *J. Med. Chem.*, 29:1561-1569], and with other diseases of retroviral origin.

              Mitsuya et al. (1985) *Proc. Natl. Acad. Sci. USA*, 82:7006-7100 reported 3'-azido-3'-deoxythymidine  
15              (AZT) blocked the replication of HIV in cultured human T lymphoblasts, and inhibited the cytopathic effects of the virus. AZT was presumably phosphorylated by the T cells and converted to the 5'-triphosphate derivative. That derivative was reported by those authors to be an  
20              inhibitor of HIV reverse transcriptase activity. Yarchoan et al. (1986) *Lancet*, i:575-580, administered AZT to patients with AIDS or AIDS-related disease complexes. The drug was reportedly well tolerated and crossed the blood/brain barrier.

25              Recently, Mitsuya et al. (1986) *Proc. Natl. Acad. Sci. USA*, 83:1911-1915 reported that the 2',3'-dideoxynucleoside derivatives of adenosine, guanosine, inosine, cytidine and thymidine also inhibited the infectivity and cytopathic effect of HIV in vitro at  
30              concentrations from 10-20 fold less than those that blocked the proliferation of uninfected T cells. These compounds were also reported to be relatively non-toxic towards host T cells. The adenosine and cytidine

derivatives were reported to be more potent than the guanosine and inosine derivatives.

5 The 2',3'-dideoxynucleosides are phosphorylated at the 5'-position in T cells to form the 5'-nucleotide triphosphate derivatives. Those derivatives are well known to be substrates for reverse transcriptase molecules. Ono et al. (1986) Biochem. Biophys. Res. Comm., 2:498-507.

10 Those 2',3'-dideoxynucleoside 5'-triphosphates are also utilized by mammalian DNA polymerases beta and gamma. Waquar et al. (1984) J. Cell. Physiol., 121:402-408. They are, however, poor substrates for DNA polymerase-alpha, the main enzyme responsible for both repair and replicative DNA synthesis in human lymphocytes.  
15 In part, these properties may explain the selective anti-HIV activity of the 2',3'-dideoxynucleosides.

Chan et al. (1982) J. Cell Physiol, 111:28-32 studied the pathways of pyrimidine nucleotide metabolism in murine peritoneal macrophages and monocytes, and  
20 reported undetectable levels of deoxycytidine kinase or thymidine kinase in these cells. High levels of adenosine kinase were found, however.

Similar high levels of adenosine kinase have been found in human monocytes and human monocyte-derived  
25 macrophages (MDM) in work carried out in the inventors' laboratory. In that preliminary work, MDM were found to exhibit about one-tenth to about one-fourth the nucleoside kinase activity of CEM T lymphoblasts (e.g. ATCC CCL 119) toward uridine, deoxycytidine and thymidine, and about  
30 two-thirds the adenosine kinase activity of CEM cells. In addition, that adenosine kinase activity of MDM cells was at least about 10-fold higher than any of the other kinase activities. Those studies also indicated relatively low levels of nucleoside phosphorylation using AZT,

dideoxycytidine (ddC) and 2',3'-dideoxyadenosine (ddA) in intact CEM T lymphoblasts and still lower levels with the MDM.

5           The ability of AZT, ddC and ddA to inhibit  
synthesis of the p24 (gag) antigen of HIV in CEM and MDM  
cells was also examined. For CEM cells, the results for  
all three compounds were similar to those discussed in  
Mitsuya et al. (1987) Nature, 325:773-778 with ddC  
10       providing the most inhibitory effect at the lowest  
concentration, followed by AZT, followed by ddA in a 3-day  
assay. Using the same concentrations (0.1-100 uM) in a  
similar 3-day assay, none of those compounds provided any  
inhibition of p24 (gag) production from MDM cells.

15           The above results explain in part the  
observations made in clinical trials with AZT. Those  
results, in part, have shown that treatment of patients  
with AIDS or AIDS-related complex with AZT has resulted in  
elevation of CD4 (T4) peripheral blood cell counts,  
20       restoration of cutaneous delayed hypersensitivity, and  
reduction of the rate of opportunistic infections and  
death; results that can be related to the effect of AZT on  
T cells.

25           However, AZT had no effect on virus isolation  
rates from peripheral blood cells. That result suggests  
that a subset of infected cells persists that represents a  
reservoir of continuing viral replication, and with the  
above work with MDM cells, indicates that macrophages  
constitute at least a portion of that in vivo reservoir of  
HIV.

30           As noted before, 2',3'-dideoxyadenosine (ddA)  
inhibits in vitro infectivity and cytopathic effects of  
HIV. Mitsuya et al. (1986) Proc. Natl. Acad. Sci. USA,  
83:1911-1915. However, ddA is a known substrate for  
adenosine deaminase (also known as adenosine

aminohydrolase, EC 3.5.4.4), which converts the compound to 2',3'-dideoxyinosine (ddI). Frederiksen (1966) Arch. Biochem. Biophys., 113:383-388. Adenosine deaminase levels in the blood cells of AIDS patients are relatively high compared to normal persons. Thus, in vivo, ddA would be expected to be degraded rapidly to 2',3'-dideoxyinosine, due to the action of endogenous adenosine deaminase. Although 2',3'-dideoxyinosine has anti-HIV activity, it is less potent than AZT or ddA (Mitsuya, 1986, above).

Several 2-substituted adenosine derivatives have been reported not to be deaminated by adenosine deaminase. For example, Coddington (1965) Biochim. Biophys Acta, 99:442-451 reported that deoxyadenosine-1-N-oxide, as well as 2-hydroxy-, 2-methyl-, 2-chloro-, 2-acetamido-, and 2-methylthio-adenosines were neither substrates nor inhibitors for adenosine deaminase. Montgomery, in Nucleosides, Nucleotides, and Their Biological Applications, Rideout et al. eds., Academic Press, New York, page 19 (1983) provides a table of comparative  $K_m$  and  $V_{max}$  data for the deamination of adenosine, 2-halo-adenosines, 2-halodeoxyadenosines and 2-fluoro-arabinoadenosine that also indicates that those 2-halo adenine derivatives are poor substrates for the enzyme relative to adenine itself. Stoeckler et al. (1982) Biochem. Pharm., 31:1723-1728 reported that the 2'-deoxy-2'-azidoribosyl and 2'-deoxy-2'-azidoarabinosyl-adenine derivatives were substrates for human erythrocytic adenosine deaminase, whereas work of others indicated 2-fluoroadenosine to have negligible activity with adenosine deaminase.

2-Chloro-2'-deoxyadenosine is phosphorylated by non-dividing (normal) human peripheral blood lymphocytes and is converted to the 5'-triphosphate. This adenine

derivative is not catabolized significantly by intact human cells or cell extracts, and is phosphorylated efficiently by T lymphocytes. Carson et al. (1980) Proc. Natl. Acad. Sci. USA, 77:6865-6869.

5           As discussed before, high levels of adenosine kinase have been found in murine peritoneal macrophages and in human monocytes. Adenosine kinase can phosphorylate 2'-deoxyadenosine derivatives, but does so less efficiently than deoxycytidine kinase. Hershfield et  
10 al. (1982) J. Biol. Chem., 257:6380-6386.

          In addition to AIDS, other infectious diseases in which pathogenic organisms persist in chronically infected monocytes/macrophages are Chagas disease and other trypanosomal diseases, Leishmaniasis, mycobacterial  
15 infections, systemic and local fungal diseases, and protozoal infections such as toxoplasmosis, malaria and pneumocystis.

          Similarly, many autoimmune diseases share common features with the pathogenesis of viral infection. The  
20 specific mechanism which mediates autoimmune disorders can be augmented by amplification systems which may involve lymphokines or humoral components.

          One form of autoimmune disease involves a cytotoxic mechanism wherein circulating autoantibody  
25 reacts with self-antigen present on a cell surface. The cytotoxic process can be mediated by complement or by cells as in antibody-dependent cell-mediated cytotoxicity. The end-result of the cytotoxic mechanism is usually cell lysis, elimination or inactivation, and this is the  
30 mechanism of many autoimmune hematologic disorders.

          A second form of autoimmune disease involves the formation of immune complexes of autoantibody plus self-antigen that can fix complement as well as initiate inflammatory processes. Organs in which such complexes



deposit are subject to inflammation, and ultimately to destruction. Nucleic acids are known to serve as antigens for this mechanism in systemic lupus erythematosus (SLE). Immune complex deposition appears to account for the glomerulonephritis present in many autoimmune disorders.

A third mechanism for autoimmune disorders is mediated by interactions of cells or their soluble products with antigen rather than with antibody and complement. This mechanism is classically manifested in delayed hypersensitivity, which is characterized by a reaction that is time-dependent, has a specific histologic sequence in terms of inflammation and cellular infiltration, and can only be transferred by cells and not by serum.

The effector mechanism of cytotoxicity can include direct cell interaction with antigen or elaboration of lymphokines and monokines. The lymphokines primarily amplify the initial reaction by nonspecifically recruiting inflammatory cells such as neutrophils and macrophages to the reaction area. At that inflammatory site, a cascade effect occurs wherein cells become activated, proliferate and secrete more cytokines.

Rheumatoid arthritis is a chronic recurrent systemic inflammatory disease primarily involving the joints. Recent studies have suggested that a virus, possibly Epstein-Barr virus, may be implicated in this autoimmune disorder. The Epstein-Barr virus is a polyclonal stimulator of B cells and can stimulate the production of rheumatoid factors by B cells. In rheumatoid arthritis, there is an increase in  $\alpha_2$ -globulin, a polyclonal hypergammaglobulinemia, and hypoalbuminemia. Cryoprecipitates of immunoglobulins are often seen in rheumatoid vasculitis.

Rheumatoid factors can be present in other autoimmune disorders, as well as in rheumatoid arthritis. Rheumatoid factors have been found to be present in some patients with systemic lupus erythematosus, Sjogren's syndrome, scleroderma and polymyositis.

The deposition of immune complexes on or in the synovia of joints appears to initiate the inflammatory response of the synovial membrane in rheumatoid arthritis. The deposited complexes fix and activate complement, which subsequently stimulates the attraction of inflammatory cells. The deeper layers of the synovium are infiltrated by both T and B lymphocytes, plasma cells, macrophages and occasionally neutrophils. The infiltrating cells elaborate several effector molecules of the inflammatory response, which transforms the joint fluid into an inflammatory exudate. The immune complexes together with the lymphocyte-released factors activate the clotting pathway leading to fibrin production and deposition in the joint space, synovium and cartilage.

Various treatment methodologies have been employed to ameliorate the symptoms of autoimmune disorders such as rheumatoid arthritis. Many of these are directed to palliative, anti-inflammatory approaches. Salicylates are commonly employed, specifically aspirin, in dosages from about 3.6 to about 5.4 grams (g) per day. Numerous side-effects are associated with high-dose aspirin therapy such as gastric upset, tinnitus and decreased platelet adhesiveness. Nonsteroidal anti-inflammatory agents, such as phenylbutazone, indomethacin, fenoprofen, ibuprofen, naproxen, sulindac, tolmetin, and mefenamic acid, and antimalarial drugs, such as chloroquine and hydroxychloroquine, have also been employed, but possess serious side effects upon prolonged usage. Other therapeutic agents such as parenteral gold

salts, penicillamine and corticosteroids also possess significant side effects.

Recently, the art has described the use of specific deoxyribosides as anti-inflammatory agents. For instance, U.S. Patent No. 4,481,197 to Rideout et al. relates to the use of unsubstituted 3-deaza-2'-deoxyadenosine derivatives in the treatment of inflammation. U.S. Patent No. 4,381,344 to Rideout et al. relates to a process for the synthesis of deoxyribosides that utilizes a bacterial phosphorylase.

A deoxyriboside derivative, 2-chloro-2'-deoxyadenosine (CdA), has been found to be an effective agent for the treatment of chronic lymphocytic leukemia and some T cell malignancies. Carson et al. (1984) Proc. Natl. Acad. Sci. U.S.A., 81:2232-2236; Piro et al. (1988), Blood 72:1069-1073. Chronic lymphocytic leukemia is a malignancy of B lymphocytes that bear the Leu-1 surface antigen.

The Leu-1 B cells represent a minor proportion of the normal pool of B lymphocytes, usually less than 20 percent. The Leu-1 B cells express surface markers that are typically found on monocytes (Mac-1 antigen) and T-lymphocytes (Leu-1 antigen). Approximately 10 percent of patients with chronic lymphocytic leukemia exhibit accompanying autoimmunity, and recently, Leu-1 B cells have been implicated in the pathogenesis of autoimmune diseases.

Phase 1 studies on humans showed that infusion of increasing doses of 2-chloro-2'-deoxyadenosine [0.1-0.5 milligrams per kilogram of body weight per day (mg/kg/day)] yielded increasing plasma concentrations of the drug [10-50 nanomolar (nM)]. Those infusions indicated that the drug was well tolerated and did not induce nausea, vomiting or fever. The dose-limiting

toxicity was bone marrow suppression, which usually occurred at doses greater than about 0.2 mg/kg/day or at plasma levels of greater than about 20 nM.

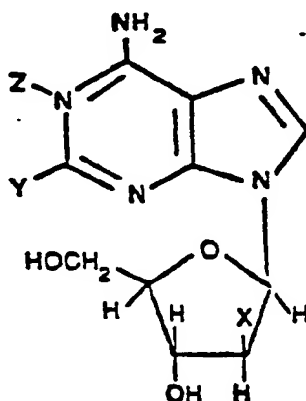
Other studies, Montgomery et al. (1959) J. Am. Chem. Soc., 82:463-468, indicated that 2-fluoroadenosine exhibits a relatively high degree of cytotoxicity. Those workers reported that C57 black mice implanted with Adenocarcinoma 755 (Ad755) could tolerate only about 1 milligram per kilogram of body weight. 2-Fluoroadenosine was found to be inactive at that level against Ad755 as well as leukemia L1210 and the Erlich ascites tumor.

Chemotherapeutic agents are described hereinafter that exhibit substantial activity toward resting lymphocytes and monocytes. These agents are also useful in the treatment of autoimmune disorders.

#### Summary of the Invention

The present invention contemplates a compound, a composition and a method for treating an infective disorder in which microorganisms reside in infected monocytes, as well as a method for the treatment of inflammation, particularly inflammation as a result of monocyte-mediated autoimmune disorders. The compound utilized in the present invention is a substituted 2'-deoxy-adenosine wherein the substituent is at the one, two and/or 2'-positions.

A compound of the invention has a structure that corresponds to that of Formula I:



I

wherein Z is O<sup>-</sup> or absent,

Y is hydrogen or a substituent containing one to about 20 atoms that is free from net ionic charge at physiological pH values, provides a soluble adenine derivative and whose presence on the adenine moiety inhibits deamination of the adenine derivative by adenosine deaminase; and

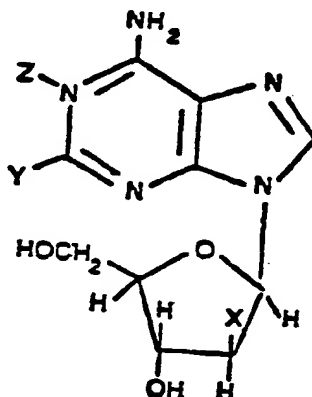
X is hydrogen or fluoro, with the provisos (i) that when Z is absent, X is fluoro; and (ii) Y is hydrogen only when Z is present and X is fluoro. Preferred Y substituents are halogen, lower alkyl, hydroxyl, lower alkylthio and lower alkanoylamido radicals.

A composition of the present invention contains a sufficient amount of one, or more, of the above compounds of Formula I, dissolved or dispersed in a pharmacologically acceptable carrier, to provide a therapeutically effective dose. Depending upon the treatment modality, the cells are contacted as discussed below with a compound of Formula I at a concentration of about 0.5 nanomolar (nM) to about 50 micromolar (uM), more preferably at about 10 nM to about 10 uM.

A method of treating a monocyte-mediated disorder is contemplated. In this method, monocytes are contacted with a composition containing a pharmacologically acceptable carrier that itself contains dissolved or dispersed therein a substituted adenine derivative having a structure that corresponds to that of Formula II as an active ingredient or agent, either alone or in combination with an antimicrobial agent. The substituted adenine derivative and the antimicrobial agent, when present, are each present in an amount sufficient to provide a therapeutically effective dose over the period of contacting. The monocytes are

contacted in vivo by administration of the composition to a mammal such as a human. In vitro contact is achieved by admixing the composition with a preparation of monocytes.

A substituted adenine derivative of Formula II has a structural formula corresponding to:



II

wherein Z is O<sup>-</sup> or absent,

Y is hydrogen or a substituent containing one to about 20 atoms that is free from net ionic charge at physiological pH values, provides a soluble adenine derivative and whose presence on the adenine moiety inhibits deamination of the adenine derivative by adenosine deaminase; and

X is hydrogen or fluoro, with the proviso that Y is hydrogen only when Z is present.

Particularly preferred compounds of Formula II are free of the Z group; i.e., Z is absent, and contain a halo group at the 2-position. Most preferred are 2-chloro-2'-deoxyadenosine and 2-chloro-2'-deoxy-2'-arafluoroadenosine.

This method of treatment decreases the level of infected monocytes in the blood as a result of the specific cytotoxicity of the compounds utilized toward monocytes. Additionally, when an antimicrobial agent is used in combination with an above compound of Formula II,

that antimicrobial agent directs its action against the causative microorganism itself.

5 In one aspect, a method of treating an infective disorder in which microorganisms reside in monocytes is contemplated. Usually, the monocytes are chronically infected. A mammal afflicted with such a microbial infective disorder is administered in vivo with a composition as discussed above.

10 A particular method of treatment for a viral infection disorder is contemplated in the present invention in which a mammal affected with a viral infection is treated with an effective therapeutic dosage of a compound of Formula II either alone or in combination with another antiviral agent, administered either together  
15 or separately, with a pharmacologically acceptable carrier. Preferred disorders for treatment are those in which the infective virus is localized in monocytes prior to cell lysis and viral release into the circulation.

20 The present invention also contemplates a method for the treatment of inflammation, particularly inflammation that occurs during autoimmune disorders, by the suppression or killing of monocytes. Here, a composition containing a compound whose structure corresponds to that of Formula II, as discussed above, is  
25 again used.

In this embodiment, a warm-blooded animal with inflammation is administered an amount of the above-described composition containing a compound of Formula II present in an amount sufficient to provide a  
30 therapeutically effective dose. Preferably, the amount is sufficient to provide a concentration in the animal's plasma of about 0.5 nanomolar (nM) to about 50 nM, more preferably of about 1 nM to about 10 nM. This method is

particularly useful for the treatment of rheumatoid arthritis in humans.

Preferably, the agent contemplated for use in the present invention is a 2-halo-2'-deoxyadenosine (2-halo-2'-deoxy-9,1'-beta-ribofuranosyladenine) or a  
5 2-halo-2'-deoxy-2'-arafluoroadenosine, and most preferably the halo group is chloro.

A further aspect contemplated by the present invention comprises the peroral administration of an  
10 effective amount of the active ingredient (agent) of the invention in a method of treating disease. Here, a compound of Formula II is utilized in which X is fluoro.

In each of the before-described methods, the substituted 2'-deoxyadenosine derivative is administered  
15 in a therapeutically effective amount. The effect of a compound of Formula II is both time and dose dependent. As a consequence, one can tailor the dosage and duration for which a particular dosage is administered to the illness being treated and the condition of the treated  
20 host mammal, such as a human. Thus, for treatment of an inflammatory disorder, impairment of monocyte function can suffice to provide relief, and an amount sufficient to provide such impairment is one measure of a therapeutically effective amount. Where the disease state  
25 or condition to be treated is more severe, or life-threatening, treatment is more aggressive, and a therapeutically effective amount is an amount that is sufficient to kill at least 50 percent of the monocytes present but is less than that which substantially impairs  
30 bone marrow function as determined by usual procedures when administration is in vivo. The monocyte killing amount of a compound of Formula II is another measure of a therapeutically effective dose and monocyte death is



measured at a time seven days after the initial administration.

The present invention has several benefits and advantages.

5           One benefit is that use of one of its methods can eliminate monocyte-borne pathogens from the body of an infected animal.

10           An advantage of the present invention is that use of one of its methods can substantially reduce inflammation caused by inflammatory diseases such as rheumatoid arthritis.

Still another advantage of the invention is that its methods can be practiced by oral administration.

15           Still further benefits and advantages of the invention will be apparent to those skilled in the art from the description that follows.

#### Brief Description of Drawings

In the drawings forming a portion of this disclosure:

20           Figure 1 is a graph showing the results of a study of the cytotoxicity of 2-chlorodeoxyadenosine (CdA) toward three cell types in the peripheral blood of seven cutaneous T-cell lymphoma patients. A continuous intravenous infusion of CdA (0.1 mg/ml in isotonic saline)  
25           was administered to each patient at a dosage of 0.1 mg/kg per day, with the patients receiving therapy for seven days. Blood samples were removed daily and cell counts performed, with averaged values being shown. Graph symbols are as follows:  $\square$  = monocytes, + = neutrophils  
30           ( $\times 10^{-1}$ ); and  $\blacklozenge$  = lymphocytes. The cell concentration (ordinate) is plotted for the day of treatment (abscissa) on which it is measured.

Figure 2 is a graph illustrating the dose-response cytotoxicity of 2-chlorodeoxyadenosine (CdA)

towards normal human monocytes ( $\square$ ), the human fibroblast cell line, GM01380, which is from normal fetal lung ( $\diamond$ ) and normal human lymphocytes ( $\blacklozenge$ ), when cultured in vitro. Cells were cultured in vitro as described in Example 2 for five days in the presence of varying concentrations of CdA from 0 to 125 nanomolar (nM), after which time viable cells were determined. The percentage of viable cells remaining after treatment (ordinate) is plotted against the concentration of CdA utilized, on a linear scale, (abscissa). The effect of the presence of deoxycytidine (dCyd 100  $\mu$ M) on CdA toxicity toward monocytes ( $\blacksquare$ ) is also illustrated.

Figure 3 is a graph of the dose- and time-dependence of 2-chlorodeoxyadenosine (CdA) cytotoxicity toward human monocytes in vitro.

Figure 4 is a graph of the dose- and time-dependence for CdA in inducing DNA strand breaks in monocytes in vitro.

Figure 5 is a graph of the biochemical effects induced in human monocytes in vitro by exposure to 1  $\mu$ M CdA over a period of 16 hours. The effects of CdA exposure upon monocyte viability ( $\square$ ), NAD content ( $\blacksquare$ ), RNA synthesis (O) and DNA strand breaks (ds-DNA;  $\bullet$ ) are illustrated.

Figure 6 is a graph that illustrates the effects of relatively low doses of CdA used to contact monocytes for a 72 hour time period. Monocyte release of interleukin-6 (IL-6;  $\blacklozenge$ ) and phagocytosis of antibody-coated red blood cells (RBC;  $\blacksquare$ ) were examined, as was the viability of the cultured monocytes ( $\square$ ). Effects are expressed as a percentage of control values versus the CdA concentration in nanomoles (nM).

Figure 7 is a graph showing the results of a study of the cytotoxicity of CdA toward monocytes and

lymphocytes of a rheumatoid arthritis patient receiving CdA therapy. A continuous intravenous infusion of CdA (0.1 mg/ml in isotonic saline) was administered at a dosage of 0.1 mg/kg per day for a five-day period.

5 Monocyte numbers are shown in open squares and lymphocyte numbers are shown as closed diamonds. Three cycles of infusions of CdA were given, and are illustrated as open rectangles for the five-day time periods of each infusion at a position in the graph above the days in the treatment  
10 regimen on which infusions were given.

#### Detailed Description of the Invention

The present invention contemplates a compound, a composition containing that compound and a method of using  
15 a composition of the invention, or another composition, for treating a monocyte-mediated disorder. It is to be understood that the monocytopenia and inhibition of monocyte function discussed hereinafter using a method of the invention were quite unexpected.

20 Those results were particularly unexpected in view of the recently published work of Urba et al. (1989) Blood, 73:38-46 and Bakul et al. (1989) Cancer, 63:14-22 who treated patients with hairy cell leukemia, another disease in which a method similar to that disclosed herein  
25 is useful. Those treatments utilized deoxycoformycin together with interferon alpha-2a, or deoxycoformycin alone, respectively.

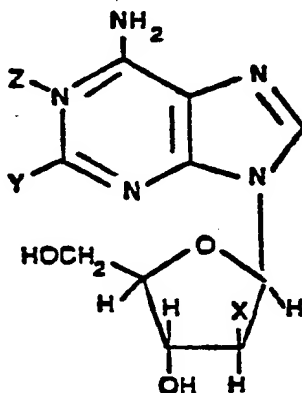
Deoxycoformycin is an irreversible inhibitor of adenosine deaminase, and its use causes adenosine and  
30 deoxyadenosine to accumulate in the cells, much the same as an adenine derivative useful herein accumulates in the cells. Lymphocytopenia and DNA strand breaks observed by the treatment are believed to be mediated by accumulation of deoxyadenosine nucleotides.

Urba et al. reported that specific lymphocytes bearing the CD4 and CD8 markers decreased during treatment. Bakul et al. studied absolute counts of cells from their patients, and reported a general decrease in numbers during treatment. Both groups, however, reported an increase in monocyte levels during treatment.

Thus, although deoxycoformycin irreversibly inhibits adenosine deaminase and permits an accumulation of adenosine and deoxyadenosine, a result similar to that which occurs by use of a method of this invention, administration of deoxycoformycin results in an increase in monocyte levels during treatment, whereas treatment using a method of this invention causes at least impairment of monocyte function or monocytopenia (monocyte death), as is discussed hereinafter. Those differences in monocyte-specific activity coming from a similar initial event, i.e., accumulation of adenosine or deoxyadenosine or a derivative as herein described, is quite startling and unexpected.

#### A. Compounds

A compound contemplated in the present invention is a substituted-2'deoxy-arabinofuranosyladenine (substituted adenine) derivative whose structure is represented by Formula I:



wherein X is either fluorine or hydrogen; Z is an oxide radical ( $O^-$ ) or is absent; and Y is hydrogen or a radical containing one to about twenty atoms that is free from net ionic charge at physiological pH values, provides  
5 a soluble adenine derivative, and whose presence on the adenine moiety inhibits deamination of the adenine derivative by adenosine deaminase, and preferably is a member of the halogen group constituted by fluorine, chlorine and bromine; with the provisos that (i) when Z is  
10 absent, X is fluoro; and (ii) Y is hydrogen only when Z is present and X is fluoro.

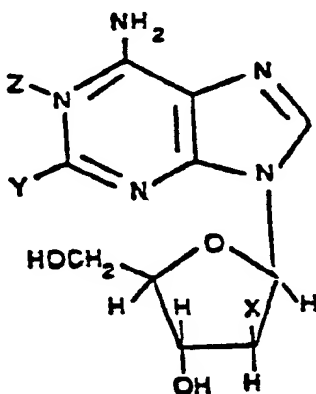
Preferably, Y is chloro. Other Y substituents are selected from the group consisting of lower alkyl, lower alkanoylamido, lower alkylthio and hydroxyl  
15 radicals. In particularly preferred embodiments, when Y is chloro, X is fluorine.

Of the compounds of Formula I, those where X is fluoro are particularly preferred for use by oral administration.

20 Illustrative of compounds of Formula I are the following arabinofuranosyl derivatives of adenine:  
2-chloro-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-adenine;  
2-bromo-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-  
25 adenine;  
2-methyl-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-adenine;  
2-fluoro-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-adenine;  
30 2-hydroxy-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-adenine;  
2-(N-acetamido)-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine;

- 2-methylthio-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine;  
2-chloro-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;  
5 2-fluoro-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;  
2-bromo-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;  
2-methyl-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;  
10 2-(N-acetamido)-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;  
2-hydroxy-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;  
15 2-methylthio-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;  
2-fluoro-9,1'-beta-D-2'-deoxyadenosine-1-oxide; and  
2-chloro-9,1'-beta-D-2'-deoxyadenosine-1-oxide.

20 A compound from a somewhat broader group of adenine derivatives is useful in a method of this invention. A compound of that broader group has a structure that is represented by Formula II:



II

wherein Z is an oxide radical ( $O^-$ ) or is absent;  
Y is hydrogen or a radical containing one to  
about twenty atoms that is free from net ionic charge at  
physiological pH values, provides a soluble adenine  
5 derivative, and whose presence on the adenine moiety  
inhibits deamination of the adenine derivative by  
adenosine deaminase; and

X is hydrogen or fluorine, with the proviso that  
Y is hydrogen only when Z is present.

10 The compounds represented by Formula I are  
included among the compounds of Formula II as are  
additional compounds. Preferred additional compounds  
included in Formula II but not in Formula I are:  
2-chloro-9,1'-beta-D-2'-deoxyribosyladenine (2-chloro  
15 deoxyadenosine);  
2-bromo-9,1'-beta-D-2'-deoxyribosyladenine;  
2-methyl-9,1'-beta-D-2'-deoxyribosyladenine  
2-fluoro-9,1'-beta-D-2'-deoxyribosyladenine;  
2-acetoamido-9,1'-beta-D-2'-deoxyribosyladenine; and  
20 2-methylthio-9,1'-beta-D-2'-deoxyribosyladenine.

Inasmuch as the X, Y and Z substituents of a  
compound of both Formula I and Formula II can be the same,  
and the compounds of Formula I are encompassed in Formula  
II, albeit the provisos that constitute portions of these  
25 formulas are different, the discussion that follows is  
intended to apply to a compound from both formulas.

It is noted that when X is hydrogen the sugar  
ring can be named as a 2'-deoxyribosyl or  
2'-deoxyarabinofuranosyl radical. Both nomenclatures are  
30 utilized herein. When the class of compounds embraced by  
Formula I or Formula II is discussed, all of the compounds  
are considered herein as derivatives of arabinose.  
However, when specific compounds of the subclass where

X = H are discussed, the more familiar deoxyribose nomenclature is used, such as in deoxyadenosine. These compounds are also referred to herein more simply as an adenine derivative.

5           In the above formulas, and in all other formulas shown herein, hydrogen atoms on the purine and furanosidyl rings that are not needed to show conformation about a particular bond are not shown. Thus, the 7-position adenine hydrogen is not shown.

10           It is also to be understood that the D isomers of compounds of the formulas are the isomers contemplated. It is further to be noted that the designation "halo" used herein is meant to include fluorine, chlorine and bromine derivatives, and to exclude iodine derivatives, which are  
15           unstable and decompose, and astatine derivatives that are radioactive. Where specific halogen derivatives are intended, those compounds are named specifically.

          As used herein, "a substituent free from net ionic charge" includes both charged and uncharged  
20           radicals, wherein when the substituent radical is charged, an internal zwitterionic charge pair is present that results in the absence of a net ionic charge for the molecule at physiologic pH values. N-oxide compounds are exemplary of such substituents.

25           As used herein, a "soluble adenine derivative" is an adenine derivative which is able to dissolve and remain soluble in a body fluid such as blood at a therapeutically effective dose as is discussed hereinafter.

30           As used herein, a "substituent whose presence on the adenine moiety inhibits deamination of an adenine derivative by adenosine deaminase" is one that, when 100 microliters of a 1 millimolar solution of the substituted adenine derivative is incubated for 3 hours at



room temperature with 25 units of calf spleen adenosine deaminase (1 unit catalyzes the deamination of 1 micromole of adenosine per minute), produces a single UV-absorbing spot upon cellulose-thin layer chromatography of the reaction mixture whose  $R_f$  value is the same as that of the substituted adenine derivative used.

The metabolism of a compound by adenosine deaminase can be investigated by the following procedure. The individual nucleosides, at concentrations from 5-200  $\mu\text{M}$  in 10 mM sodium phosphate, pH 7.5, are incubated at 18-20 degrees C with 0.01 EU/ml calf intestinal adenosine deaminase. The change in the optical density at 265 nm and 250 nm is monitored spectrophotometrically. The  $K_m$  and  $V_{max}$  values are determined by the Lineweaver-Burke method, utilizing the  $\Delta E_{265}^M$  between adenosine and inosine.

The ratio  $V_{max}/K_m$  also provides a measure of relative efficiency of deamination by the enzyme. A substituent that provides a  $V_{max}/K_m$  ratio that is about 1 percent or less than that for the ratio obtained using 2'-deoxyadenosine is also a "substituent whose presence on the adenine moiety inhibits deamination of an adenine derivative by adenosine deaminase."

As used herein, lower alkyl radicals include  $C_1$ - $C_6$  straight chain, branched and cyclic alkyl groups, for example, methyl, ethyl, n-butyl, t-butyl, n-hexyl, 1-ethylbutyl, cyclopentyl, cyclohexyl and the like. Lower alkanoylamido radicals include  $C_1$ - $C_6$  radicals, for example, formamido, acetylamido, propionamido, hexamoylamido and the like. Lower alkylthio radicals include  $C_1$ - $C_6$  straight chain, branched and cyclic alkyl groups as discussed above linked to a thio radical.

The pharmacologically acceptable salts of a compound of Formula I or Formula II are also utilized.

The phrase "pharmacologically acceptable salts", as used herein, refers to non-toxic acid addition salts that are generally prepared by reacting a compound with a suitable organic or inorganic acid. Representative salts include the hydrochloride, hydrobromide, sulfate, phosphate, citrate, acetate, maleate and the like.

#### B. Compositions

A compound of Formula I dissolved or dispersed in or together with a pharmacologically acceptable carrier constitutes a composition of this invention. However, since a compound of Formula I is embraced by Formula II, and a composition containing a compound of Formula II is useful in a method of the invention, a composition containing a compound of Formula I will frequently be discussed hereinafter in terms of a composition of a compound of Formula II.

A compound of Formula II and its pharmacologically acceptable salts are useful in both short and long term treatment. For instance, a 2-substituted-9,1'-beta-2'-deoxy-2'-fluoro-D-arabino-furanosyladenine is administered to the warm-blooded animal internally, e.g., parenterally, orally, or rectally as a suppository, in an effective amount.

Although a compound of Formula II and its pharmacologically acceptable salts can be administered as the pure chemical, it is preferred that it be administered as a pharmaceutical composition. In either event, it is administered in an amount sufficient to provide a therapeutically effective dose as is discussed hereinafter.

Accordingly, the present invention utilizes a pharmaceutical composition comprising a therapeutically effective dose of a compound of Formula I or Formula II, preferably wherein X is fluoro, or a pharmacologically

acceptable salt thereof, hereinafter referred to as the "active ingredient" or "agent", dissolved or dispersed in a pharmacologically acceptable carrier or diluent.

5 A pharmaceutical composition is prepared by any of the methods well known in the art of pharmacy all of which involve bringing into association the active compound and the carrier therefor. For therapeutic use, a compound utilized in the present invention can be administered in the form of conventional pharmaceutical  
10 compositions. Such compositions can be formulated so as to be suitable for oral or parenteral administration, or as suppositories. In these compositions, the agent is typically dissolved or dispersed in a physiologically tolerable carrier.

15 A carrier or diluent is a material useful for administering the active compound and must be "pharmacologically acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof. Thus, as  
20 used herein, the phrases "physiologically tolerable" and "pharmacologically acceptable" are used interchangeably and refer to molecular entities and compositions that do not produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when  
25 administered to a mammal. The physiologically tolerable carrier can take a wide variety of forms depending upon the preparation desired for administration and the intended route of administration.

30 As an example of a useful composition, a compound of Formula II can be utilized in liquid compositions such as sterile suspensions or solutions, or as isotonic preparations containing suitable preservatives. Particularly well-suited for the present purposes are injectable media constituted by aqueous

injectable isotonic and sterile saline or glucose solutions. Additional liquid forms in which these compounds can be incorporated for administration include flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, peanut oil, and the like, as well as elixirs and similar pharmaceutical vehicles.

The agents can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain stabilizers, preservatives, excipients, and the like in addition to the agent. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic.

Methods to form liposomes are known in the art. See, for example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 et seq.

An agent of Formula II can also be used in compositions such as tablets or pills, preferably containing a unit dose of the compound. To this end, the agent (active ingredient) is mixed with conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate, gums, or similar materials as non-toxic, physiologically tolerable carriers. The tablets or pills can be laminated or otherwise compounded to provide unit dosage forms affording prolonged or delayed action.

It should be understood that in addition to the aforementioned carrier ingredients the pharmaceutical

formulation described herein can include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, flavoring agents, binders, surface active agents, thickeners, lubricants, preservatives (including antioxidants) and the like, and substances included for the purpose of rendering the formulation isotonic with the blood of the intended recipient.

The tablets or pills can also be provided with an enteric layer in the form of an envelope that serves to resist disintegration in the stomach and permits the active ingredient to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, including polymeric acids or mixtures of such acids with such materials as shellac, shellac and cetyl alcohol, cellulose acetate phthalate, and the like. A particularly suitable enteric coating comprises a styrene-maleic acid copolymer together with known materials that contribute to the enteric properties of the coating. Methods for producing enteric coated tablets are described in U.S. Patent 4,079,125 to Sipos, which is herein incorporated by reference.

The term "unit dose," as used herein, refers to physically discrete units suitable as unitary dosages for administration to warm blooded animals, each such unit containing a predetermined quantity of the agent calculated to produce the desired therapeutic effect in association with the pharmaceutically acceptable diluent. Examples of suitable unit dosage forms in accord with this invention are tablets, capsules, pills, powder packets, granules, wafers, cachets, teaspoonfuls, dropperfuls, ampules, vials, segregated multiples of any of the foregoing, and the like.

Oral administration of the compound is a particularly attractive mode of administration. One

drawback usually associated with oral administrations of bioactive nucleoside compounds, however, is their potential decomposition in the acidic conditions of the stomach. That is, the glycosidic bond tends to hydrolyze under acid conditions.

However, where oral administration is desired, substitutions on the 2-position of the adenine ring of the compound of Formula II are utilized along with a 2'-fluoro-substituted arabinofuranosidyl ring.

Marquez et al. (1987) Biochem. Pharm., 36:2719-2722 reported preparation of 2'-fluoro-2',3'-dideoxyribose and 2'-fluoro-2',3'-dideoxyarabinose derivatives of adenine. Their findings stated that both derivatives were stable at a pH value of 1 at 37 degrees C., whereas dideoxyadenosine had a half-time of 35 seconds under those conditions.

The ability of an adenine derivative to be or not to be a substrate for adenosine deaminase is more a function of the 2-substituent or lack thereof on the adenine portion of the molecule than a function of substituents on the linked sugar ring portion, at least as far as the substituents on both rings herein are concerned.

#### C. Methods

As noted earlier, a method of treating a monocyte-mediated disorder is contemplated herein. Broadly in that method, monocytes are contacted with a composition containing a pharmacologically acceptable carrier having dissolved or dispersed therein, as an active ingredient, a substituted adenine derivative (substituted 2'-deoxy-adenosine) whose structure corresponds to that of previously discussed Formula II. That agent of Formula II can be present alone or in combination with an antimicrobial agent, as a second

active ingredient (agent). The substituted adenine derivative, and the antimicrobial agent when present, are each present in the composition in an amount sufficient to provide a therapeutically effective dose over the period of contacting.

It is particularly contemplated that contact between the monocytes and agent of a composition be in vivo. However, in vitro contact as is illustrated hereinafter and as can be achieved by well known extracorporeal methods are also contemplated.

The phrase "monocyte-mediated" is used herein to mean that monocytes or cells of the monocyte lineage such as macrophages are involved in the disease or condition, collectively referred to as the "disorder", to be treated. The degree of monocyte involvement in a given disorder is a function of that disorder and can be different for different types of disorders. For example, in the case of microbial (bacterial, parasitic, viral, and the like) disease, the monocytes harbor the microbes and can shield them from treatment with usual drugs. In the case of inflammatory disorders such as arthritis, the monocytes and/or macrophages accumulate at the site of inflammation, and contribute to the disorder through one or more mechanisms such as phagocytosis, and release of hydrolytic enzymes and cytokines, such as IL-6, release of fever-inducing protein, such as IL-1, and walling-off of the inflamed area.

A method for the treatment of infective disorders in which microorganisms such as viruses, bacteria, parasites, or the like reside in chronically infected monocytes is contemplated as a specific embodiment of the present invention. The treatment of viral infections, and especially T-lymphotropic viral infections and disorders in which the infective virus is

localized in monocytes of an affected mammal is a particular aspect of the present invention. Such affected mammals are preferentially human patients.

5        Thus, a composition containing a compound of Formula II is administered either alone or in combination with an antimicrobial agent to a mammal affected with such a microbial disorder in amounts sufficient to provide a therapeutically effective dose of each drug to the mammal. The antimicrobial agent is administered to the mammal  
10        either together with or separately from the administration of the composition containing a compound of Formula II. The composition is maintained within the mammal until its constituent components are eliminated by usual bodily processes.

15        The amount of a compound of Formula II present in a composition and used in a method as described above is a function of several variables, as is well known in the medicinal arts. Among those variables are whether the administration is in vivo or in vitro, if in vitro, the  
20        number of cells to be treated that are present, the animal treated, the disease to be treated in the animal or cells, and also the method of administration. Exemplary concentrations are illustrated hereinafter for both in vivo and in vitro uses.

25        Regardless of the above variables, in disorders where a microorganism is known to be involved such as those discussed before and immediately hereinafter (non-inflammatory disorders or those other than autoimmune-related disorders) the substituted 2'-deoxyadenine  
30        derivative is administered in an amount that is sufficient to kill at least about 50 percent of the monocytes present over the duration of the treatment. Preferably, about 90 to 100 percent of the originally present monocytes are killed.



When the administration is in vivo, the amount administered is less than that which substantially impairs bone marrow functions as determined by usual procedures.

When the administration is in vitro as in an  
5 extracorporeal administration to an animal such as a human where the 2'-deoxyadenine derivative does not substantially enter the body of the treated animal, a limiting concentration is that which is not prohibitively cytotoxic to other cells that may be present.

10 An amount sufficient to kill at least about 50 percent of the monocytes originally present while not substantially impairing bone marrow function over the course of the administration of the agent is one way of defining a therapeutic dose.

15 The above amount of a 2'-deoxyadenine derivative of Formula II or its pharmacologically acceptable salt present in the composition is also an amount sufficient to provide about 0.04 to about 0.20 mg/kg of body weight of the treated host mammal per day, more preferably about  
20 0.05 to about 0.15 mg/kg/day, and most preferably about 0.1 mg/kg/day, when given in vivo. This amount is another way of defining a therapeutically effective dose that is particularly useful when a compound of Formula II is administered by infusion.

25 The molar plasma concentration of the compound of Formula II or the pharmacologically acceptable salts thereof during treatment is preferably in the range of about 1 nanomolar (nM) to about 100 nM, particularly about 5 nM to about 50 nM, and more preferably about 10 nM to  
30 about 20 nM. Molarity of the 2'-deoxyadenine derivative in plasma of the treated (administered to) animal thus provides still another measure of a therapeutically effective dose from which the amount in a composition can be calculated.

It is to be understood that the above therapeutically effective dosages need not be the result of a single administration, and are usually the result of the administration of a plurality of unit doses. Those  
5 unit doses can in turn comprise portions of a daily or weekly dosage, and thus, the therapeutically effective dose is determined over the period of treatment (contacting).

Oral administration is the preferred mode of  
10 administration for the 2'-fluoroadenine derivatives, as already noted. To achieve the desired plasma concentration of the agent, a range of doses can be employed depending upon the specific mode of administration, objective of the particular treatment, the  
15 particular compound being used, and like considerations.

For example, for oral administration, the daily dose can be about 0.04 to about 0.20 mg/kg of body weight, more preferably about 0.05 to about 0.15 mg/kg/day, and most preferably about 0.1 mg/kg body weight. In general,  
20 the amount of active substituted adenine derivative administered can vary over a relatively wide range to achieve, and preferably maintain, the desired plasma concentration.

Unit dosage forms of the adenine derivative can  
25 contain about 0.1 milligrams to about 15 milligrams thereof. A preferred unit dosage form contains about 0.1 to about 1 milligram of agent and can be administered 2 to 5 times per day. However, it should be noted that continuous infusion at a rate designed to maintain the  
30 above described plasma concentration is also contemplated.

Duration of a particular treatment can also vary, depending on severity of the disease, whether the treatment is intended for an acute manifestation or for prophylactic purposes, and like considerations. Typical

administration lasts for a time period of about 5 to about 14 days, with a 7-day time course being usual. Courses (cycles) of administration can also be repeated at monthly intervals, or parenteral unit dosages can be delivered at weekly intervals. Oral unit dosages can be administered at intervals of one to several days to provide the determined therapeutically effective dose. Thus, in vivo administration of a before-discussed dosage over a time period of about 5 to about 14 days or at weekly or daily intervals provides an amount sufficient to kill at least about 50 percent of the originally present monocytes.

This method of treatment produces a decrease in the level of monocytes in the blood due to the toxicity of the utilized compounds of Formula II toward monocytes. This method can be used to reduce the number of monocytes circulating in a treated mammal's blood stream by about 90 percent of the number present prior to treatment over a seven day treatment period with the level of circulating monocytes returning to pretreatment levels about two weeks after the treatment stopped. This exemplary study is illustrated hereinafter.

The combination therapy methodology of the present invention focuses therapeutic agents against both the causative infective agent and the monocyte host. Examples of particular diseases that are amenable to this treatment (and parenthesized therapeutic agents currently utilized in their treatment) in humans are the following:

|                |   |
|----------------|---|
| Chagas disease | (nifurtimox)  |
| Leishmaniasis  | (stibogluconate;<br>amphotericin B;<br>pentamidine isethionate) |

34

|   |               |  |
|---|---------------|--|
|   | Toxoplasmosis | (pyrimethamine;<br>sulfonamide)                                  |
| 5 | Malaria       | (chloroquine; primaquine;<br>pyrimethamine, mefloquine)          |
|   | Pneumocystis  | (trimethoprim-sulfamethoxa-<br>zole; pentamidine<br>isethionate) |

10

Therapeutic amounts and treatment regimens for the above-noted parenthesized therapeutic agents are well known, and can be readily obtained from usual sources such as the Physicians Desk Reference, 42 ed, Medical Economics Corp., Oradell, N.J. (1988).

15

In an exemplary embodiment, a patient afflicted with a Leishmaniasis is treated in the method of the present invention. The patient receives a composition containing a compound of Formula II at a therapeutically effective dose, together with the administration of pentamidine. In a particularly preferred embodiment, the patient is perorally administered a composition containing 0.15 mg/kg/day of 2-chloro-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine in a pharmacologically acceptable carrier, and receives 4 mg/kg/day of pentamidine by intramuscular injection, for a period of about 7 days.

20

25

Other diseases of uncertain etiology wherein the monocyte/macrophage is believed to be involved are sarcoidosis, chronic granulomatous hepatitis, Wegener's granulomatosis, Paget's disease, atherosclerosis, inflammatory bowel disease, and granulomatous uveitis. These diseases are currently treated principally with steroids, such as prednisone, or etidronate (Paget's disease) or sulfasalazine (granulomatous uveitis).

30

In the treatment of a viral infection, human patients or lower animals such as laboratory animals like mice, rats, chimpanzees and the like, and veterinary animals such as dogs, cats, cattle, sheep, pigs and the like are administered a composition containing a compound of Formula II either alone or in combination with another antiviral therapeutic agent in amounts sufficient to provide therapeutically effective doses of one or both drugs, as is applicable.

In an exemplary treatment methodology, patients affected with AIDS are treated with a combination of azidothymidine (AZT), dideoxycytidine (ddC), interferon, acyclovir and the like, and a substituted-2'-deoxyarabinofuranosyladenine derivative such as 2-chlorodeoxyadenosine (CdA) or 2-methyl-9,1'-beta-2'-deoxy-2'-fluoro-D-arabino-furanosyladenine. AZT combination therapy has recently been utilized in the treatment of AIDS, wherein lower dosages of AZT are administered together with reduced dosages of a second chemotherapeutic agent, as detailed in AIDS/HIV Experimental Treatment Directory, Vol 2 [American Foundation for AIDS Research (1988)]. For example, the patients receive a therapeutically effective dosage of AZT (about 200 mg orally every four hours) for four weeks, together with about 0.04 to about 0.20 mg/kg of body weight per day of CdA for about 5 to about 14 days, and typically for about 7 days.

The administration of the CdA (or an analogous compound of Formula II) produces a dramatic decrease in blood monocytes, in which the virus is harbored, as a result of the compound's enhanced toxicity towards monocytes. The concomitant administration of AZT (or another antiviral agent) kills viruses (or inhibits viral replication) by entering cells that are invaded by the

viruses and presumably incorporating into growing DNA chains resulting in termination of the chains and subsequent inhibition of viral replication within these cells and further infection.

5           The combination treatment methodology of the present invention is thus effective against both active virus that invades cells and latent virus present in monocyte host cells. Additional therapeutic agents for treating HIV and appropriate dosages of administration can  
10 be found in AIDS/HIV Experimental Treatment Directory, above.

          By the term "inflammation" is meant the reactive state of hyperaemia and exudation from its blood vessels, with consequent redness, heat, swelling and pain, which a  
15 tissue enters in response to physical or chemical injury or bacterial invasions. Such inflammations are mediated by monocytes, and other phagocytes.

          Clinical conditions with which monocyte-mediated inflammation is associated, and hence for which an anti-  
20 inflammatory agent is indicated, include, for example, arthritis, including rheumatoid arthritis and osteoarthritis, post-operative inflammation, dental inflammation, and acute and chronic ocular inflammatory diseases such as conjunctivitis.

25           There is provided as a further aspect of the present invention a method for the treatment of inflammation, particularly that which occurs during autoimmune disorders. The method comprises administration of a composition as previously described in an amount  
30 sufficient to provide a therapeutically effective dose of a compound of Formula II or a pharmacologically acceptable salt thereof. Preferably, that administration is by the oral route and X is fluoro.

A dosage over a time period described previously for non-autoimmune-related conditions can also be used for treatment of inflammation. Such a treatment although effective is, however, quite aggressive and can leave the treated host animal in an unnecessarily immunocompromised state.

A less aggressive treatment regimen is also therefore contemplated. Here, a before-described dosage, e.g., plasma concentration is again utilized, but for a shorter contact time course so that monocyte function is impaired, but the monocytes are not substantially killed as is the result of the before-discussed treatment regimen. Impairment of monocyte function is herein defined as a reduction of at least about 25 percent in the spontaneous secretion of interleukin-6 (IL-6) by monocytes cultured in the presence of a compound of Formula II for a time period of 72 hours. A useful assay for monocyte impairment is discussed hereinafter.

In an exemplary treatment regimen, a compound of Formula II is administered in an amount of about 0.04 to about 0.20 mg/kg/day, more preferably about 0.05 to 0.15 mg/kg/day, and most preferably about 0.1 mg/kg/day to provide a plasma concentration of about 1 nM to about 100 nM, and more preferably about 5 nM to about 20 nM. That single administration is repeated weekly over a time period of several months, e.g., about three to about nine months.

Such an administration can be carried out on an out-patient basis for humans using an intravenous infusion lasting about 2 to about 4 hours in a doctor's office. As such, the treatment is far less invasive than is a continuous infusion over a period of several days that usually requires a hospital stay for the host mammal; i.e., human patient.

Conditions where the suppression of the immune response is desirable include autoimmune diseases such as systemic lupus erythematosus, hemolytic anemia, ulcerative colitis, nephrosis and the prevention of rejection of foreign cells such as grafts including organ transplants.

#### D. Compound Synthesis

A compound useful herein where Z is absent can be prepared by condensing an appropriately substituted adenine directly with an appropriately substituted sugar ring as by the techniques described in Montgomery et al., (1986) J. Med. Chem., 29:2389-2392, by the method taught in U.S. Patent No. 4,082,911, or as described in the citations of Herdewijn et al. (1987) J. Med. Chem., 30:2131-2137, which disclosures are incorporated herein by reference. An appropriately substituted adenine can be prepared by following reported literature syntheses or analogous syntheses. Still further, Wright et al. (1987) J. Org. Chem., 52:4617-4618 recently prepared 2-chloro- and 2-bromo-2'-deoxyadenosines by direct reaction of the appropriate 2,6-dihalo purine with a 3',5'-protected-alpha-1-chlororibose using sodium hydride in acetonitrile, followed by treatment with methanolic ammonia at 60 degrees C to deprotect the resulting 3',5'-hydroxyls and form the 6-amino group of the finally produced adenosine. Fukukawa et al. (1983) Chem. Pharm. Bull., 31(5):1582-1592 also report syntheses of 2'-deoxy-2'-arabalo-substituted adenosine derivatives.

The 2'-deoxy-2'-fluoro-arabinofuranosyladenine compounds of the present invention are produced as described hereinafter in the Examples. The synthesis is similar to that taught in Marquez et al. (1987) Biochem. Pharmacol., 36:2719-2722, herein incorporated by reference, in which 6-chloropurine is condensed with 3-O-acetyl-5-O-benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl



bromide. The functionalized halosugar is produced according to the method reported by Reichman et al. (1975) J. Carbohyd. Res., 42:233 and the 2'-deoxy-2'-fluoro-arabinofuranosyladenine compound is obtained by ammonolysis with concentrated methanolic ammonia which removes the protective groups.

The adenosine-1-N-oxide group of compounds, i.e., where Z is present, is of particular interest since those materials, per se, are most likely not incorporated into a growing polynucleotide chain because the presence of the N-oxide group probably interferes with hydrogen bonding during that synthesis. Rather, it is believed that the N-oxide compounds are reduced by an endogenous reductase prior to their incorporation into and termination of the growing chain.

Nevertheless, being free from a net ionic charge, but possessing an internal zwitterionic charge pair, the N-oxide compounds can penetrate cell membranes. Those compounds are also somewhat more water-soluble than are the corresponding un-oxidized compounds.

Without wishing to be bound by theory, it is nevertheless believed that the N-oxide compounds enter the cell and are phosphorylated, in keeping with the report of such phosphorylation in Lindberg et al. (1967) J. Biol. Chem., 242:350-356. A pool of such derivatives is maintained intracellularly until such time as the N-oxide function is reduced and the nucleotide is incorporated to terminate the appropriate, growing polynucleotide chain.

The 1-N-oxide compounds are readily prepared by the method of Klenow et al. (1961) Biochim. Biophys. Acta, 52:386-389, with slight modification, as discussed hereinafter.

The present invention is further illustrated by the following examples which are not intended to limit the scope of the invention in any way.

5      **EXAMPLE 1:      Cytotoxicity of CdA In Vivo**

10      The effect of 2-chlorodeoxyadenosine (CdA) administration upon the level of circulating peripheral blood monocytes, lymphocytes, neutrophils and platelets was determined as follows.

15      Seven cutaneous T-cell lymphoma patients were administered continuous intravenous infusion of a composition containing 2-chlorodeoxyadenosine at a dosage of 0.1 mg/kg of body weight per day in isotonic saline. Blood samples were obtained daily and the number of viable cells present were enumerated daily for seven days after treatment.

20      The average results obtained as to three cell types are illustrated in Figure 1, which shows the enhanced toxicity of 2-chlorodeoxyadenosine toward blood monocytes. The mean level of monocyte decrease caused by contacting the monocytes with the CdA-containing composition at day 7 was 80%. Monocytes disappeared completely from the circulation of five of the seven lymphoma patients by the fifth treatment day.

25      Platelet and hemoglobin levels were constant over the time period shown. As is seen, no significant decrease in the number of circulating neutrophils was noticed, whereas the number of circulating lymphocytes decreased by 30% by the end of period of infusion of CdA. Monocyte numbers returned to approximately the pre-administration values within about two weeks after cessation of the CdA administration.

Thus, the present invention provides a method for decreasing the number of circulating blood monocytes. Similarly, it has been found that administration of 2-chlorodeoxyadenosine to autohemolytic anemia patients resulted in a significant decrease in autoantibody production and concomitant reduction in hemolysis.

EXAMPLE 2: Cytotoxicity of CdA In Vitro

Comparative cytotoxicity of 2-chlorodeoxyadenosine (CdA) to purified human monocytes, lymphocytes and human fibroblast cells was determined as follows.

Peripheral blood monocytes and lymphocytes were isolated by well known methods from normal subjects. The cells were cultured while fresh at a density of about  $5 \times 10^5$  cells per ml in 96-well flat-bottomed tissue culture plates using a contacting composition containing RPMI 1640 medium supplemented with 2 mM L-glutamine, 50 uM 2-mercaptoethanol and 20% autologous plasma (complete medium), and further containing varying concentrations (0-125 nanomolar) of CdA over a five day culture period at a temperature of 37 degrees C in air containing 5% CO<sub>2</sub>. The human fibroblast cell line GM01380 was obtained originally from the NIGMS Human Genetic Mutant Cell Repository, Camden, NJ, and cultured under the same conditions as above. That cell line was from a normal fetal lung.

Toxicity towards the monocytes and fibroblasts was determined by a modification of the MTT reduction assay described in Mosmann (1983) J. Immunol. Meth., 65 55-63. After culturing for up to five days, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) at 0.2 mg (40 ul) was admixed with each well and the

incubation was continued for another 4 hours. The plates were then centrifuged at 1000Xg for 10 minutes, and supernatants were carefully aspirated with a finely drawn pipette.

5           Acidified isopropanol (100 ul; using 0.04 N HCl) was added to each well. The plates were sealed, shielded from light, and placed at -20 degrees C for about 18 hours to permit complete dissolution of the blue formazan precipitates.

10           Viable cell numbers were determined using a Dynatech microplate spectrophotometer, measuring absorbance at a wavelength of 570 nanometers (nm), using a reference wavelength of 630 nm. The assay could detect as few as 2000 unactivated monocytes, and the absorbance of  
15           MTT formazan at 570 nm was linear with respect to the number of monocytes over a range of about  $2.5-20 \times 10^3$  cells.

          Toxicity of CdA towards cell lines and lymphocytes in suspension was determined by erythrosin B  
20           dye exclusion using standard techniques.

          Results of this in vitro cytotoxicity assay are shown in Figure 2. Cytotoxicity is expressed as a percentage of viable cells remaining after five days of exposure to CdA in comparison with the number of viable  
25           cells after five days without any CdA added to the culture medium (VIABLE CELLS % OF CONTROL). Whereas 50% of the cultured monocytes were killed after five days exposure to 20 nM CdA, no appreciable toxicity was observed on the growth of fibroblast cells at the same CdA concentration.  
30           It was further noticed that monocyte sensitivity to CdA was substantially reduced by the presence of deoxycytidine (100 uM). This result appears to implicate deoxycytidine kinase activity in the cytotoxicity of CdA.

It should be emphasized that these results were unexpected. It has been found that the toxicity of CdA depends upon its phosphorylation and conversion to CdA-5'-triphosphate. The formation of CdA nucleotides is a function of the ratio between deoxycytidine kinase activity and 5'-nucleotidase activity. Because human macrophages were reported to have low deoxycytidine kinase levels, and appear to have ample 5'-nucleotidase, cells of the monocyte/macrophage lineage were not expected to exhibit sensitivity to CdA. Importantly, the concentrations of CdA that are toxic to monocytes in vitro are in the same range as those measured in the plasma of patients who currently receive CdA chemotherapy for chronic lymphoid malignancy.

Furthermore, because cells of the monocyte/macrophage lineage are responsible, in large part, for inflammatory responses, the results of this study indicate that compounds of Formula II can be used to selectively reduce the number of circulating, blood monocytes and thereby ameliorate inflammation.

EXAMPLE 3: In Vitro Cytotoxicity of  
CdA Toward Monocytes

Freshly isolated human monocytes were cultured at a concentration of  $10^5$  cells/well in 96-well flat-bottom tissue culture plates. The cells were incubated in complete medium (Example 2) for about 12 hours following initial plating. The cells were then treated by the addition of various concentrations of CdA to form monocyte-containing compositions specific wells and the plates were incubated, as discussed previously.

The percentage of viable cells that were present in wells containing the treated monocytes was determined

daily for six days. The results are illustrated in Figure 3, which shows that CdA is toxic to monocytes and produces a significant decrease in cell viability within 2 days of treatment with 50 nM CdA.

5

EXAMPLE 4: DNA Damage in Monocytes Exposed to CdA

Monocytes were plated as discussed previously, and were then contacted with compositions containing various concentrations of CdA. The amount of DNA damage in monocytes exposed to CdA was determined by the fluorescent assay for DNA unwinding in alkaline solution described by Birnboim and Jevcak (1981) Cancer Res., 41:1889-1892, modified to accommodate lower cell numbers (Thierry et al. (1985) Radiation Res., 102:347-358).

The unwinding rate of DNA in alkaline solution at 15 degrees C is proportional to the number of DNA strand breaks or alkali-labile sites. The ethidium bromide fluorescence of residual duplex DNA in samples exposed to pH 12.8 for one hour was compared to the fluorescence of a DNA aliquot that was not exposed to alkali. The percent residual double-stranded DNA at 1 hour was taken as a measure of the DNA damage in the sample. The results are illustrated in Figure 4.

DNA breaks appeared within 2 hours in human monocytes exposed to 10 nM CdA, and accumulated with time during CdA exposure. The level of DNA damage was dose-dependent.

EXAMPLE 5: Biochemical Effects of CdA in Human Monocytes

Cellular NAD content was measured in human monocytes following incubation in culture with CdA (1 uM).

An alcohol dehydrogenase cycling assay as described in Jacobson and Jacobson (1976) Arch. Biochem. Biophys. 175:627-634 was used to measure NAD.

Monocytes, cultured and contacted as before described, were detached from the culture wells and were treated with perchloric acid (0.5 M) for 10 minutes at 4 degrees C. The mixture was clarified and neutralized with KOH containing potassium phosphate buffer (0.33 M) at pH 7.5. Monocyte ATP was quantitated in perchloric acid extracts by anion exchange HPLC, using a Whatman SAX column with an isocratic mobile phase consisting of  $\text{KH}_2\text{PO}_4$  (0.25 M), KCl (0.5 M) and acetonitrile (2%) at pH 3.6 following the procedures of Carson et al. (1980) Proc. Nat'l. Acad. Sci. USA, 77:6865-6869.

NAD<sup>+</sup> consumption for poly(ADP-ribose) synthesis is a known consequence of severe DNA damage in eukaryotic cells. To determine the potential role of NAD depletion in the marked toxicity of CdA towards monocytes, the temporal changes in oxidized NAD and ATP in cells exposed to CdA at 1  $\mu\text{M}$  were studied.

Figure 5 shows the changes in oxidized NAD in monocytes exposed to CdA. In contrast to measures of DNA integrity [double-stranded (ds)-DNA], the monocyte NAD content remained relatively constant during the first four hours of exposure, (> 95% of control NAD), but declined progressively thereafter. The fall in NAD preceded the decreases in ATP and in cell viability that was first evident after a 16 hour exposure to CdA.

Monocyte RNA synthesis following CdA exposure was studied by measuring the incorporation of <sup>3</sup>H-uridine. Monocytes were exposed to <sup>3</sup>H-uridine (20  $\mu\text{Ci}/10^6$  cells) during the final 1 hour of CdA (1  $\mu\text{M}$ ) exposure. Radioactivity measured was contained in trichloroacetic acid precipitates collected onto cellulose acetate filters

and measured by liquid scintillation counting. Figure 5 illustrates that 1 uM CdA caused a progressive reduction in RNA synthesis that was detectable after the first hour of culture, and was coincident with the appearance of DNA damage.

EXAMPLE 6: Monocyte Function Assays

The effects of sub-lethal CdA concentrations on monocyte function in vitro was also studied. Cells were contacted and cultured with CdA (5-20 nM) for three days, after which phagocytosis and supernatant IL-6 activity were assayed. Figure 6 shows that despite unchanged cell viability, the phagocytosis of antibody-coated erythrocyte targets was markedly suppressed by 10 and 20 nM CdA.

The ability of dialyzed culture supernatants to promote proliferation of B9.9 hybridoma cells is a measure of IL-6 activity (Helle et al.(1988) Eur. J. Immunol., 18:1535-1540). Monocytes spontaneously secrete IL-6 upon adherence to plastic during short-term in vitro culture (Guerne et al. (1989) J. Clin. Invest., 93:585-592). Monocytes cultured in autologous plasma 20% for three days secreted approximately 18 U per ml of IL-6, as determined by the bioassay standard curve using rIL-6.

Figure 6 also shows that contacting monocytes with sub-toxic concentrations of CdA for three days inhibited the spontaneous secretion of IL-6 into the culture supernatant. At cytotoxic concentrations of CdA, however, supernatants contained high levels of IL-6, presumably due to cell lysis and release of the monokine from intracellular stores.

Monocyte phagocytosis was assayed using autologous erythrocyte targets, sensitized with a subagglutinating titer or rabbit anti-human erythrocyte



IgG (Cappel, Malvern, PA). Monocytes ( $2 \times 10^5$  cells/well) were exposed to sub-toxic concentrations of CdA in microwell plates for 72 hours. Complete medium was replaced with medium containing 10% fetal bovine serum, and a suspension of sensitized erythrocytes (0.25% packed cell volume) was added to the adherent monocyte layers. After incubation for 4 hours at 37 degrees C, the degree of phagocytosis was quantitated by the spectrophotometric method of Jungi (1985) J. Immunol. Meth., 82:141-153.

This assay is based on the hemoglobin-catalyzed peroxidation of diaminobenzidine by detergent lysates of mononuclear phagocytes.

The spontaneous secretion of IL-6 by cultured monocytes was measured by the hybridoma growth factor bioassay as described by Guerne et al. (1989) J. Clin. Invest., 83:585-592. In this assay, the proliferation of a B9.9 murine hybridoma subclone is dependent on the presence of IL-6. Supernatants collected from monocytes cultured up to 72 hours with CdA were first dialyzed to remove the drug, then diluted 1:12 into wells containing B9.9 cells from the IL-6 bioassay. In order to eliminate the stimulatory effects of contaminating lipopolysaccharide during monocyte isolation, polymyxin B (12.5 ug/ml) was added to reagents, buffers, and adherence media used to prepare cells for these studies.

EXAMPLE 7: Cytotoxicity of CdA In Vivo

A study similar to that described in Example 1 was carried out with three patients having rheumatoid arthritis. In this study, a composition containing CdA as discussed in Example 1 was administered to patients by infusion for a five-day time period in three cycles using about four to six weeks between treatment cycles.

Data for monocyte and lymphocyte cell numbers are shown in Figure 7 for patient 1, a 63 year old woman with sero-positive rheumatoid arthritis. Neutrophil and platelet numbers were also assayed and were shown to be substantially constant throughout the one hundred days of the study. Similar results for three of the four cell types were obtained for patients 2 and 3. Patient 3 had a neutropenia temporally related to a viral syndrome, but which resolved after discontinuation of non-steroidal anti-inflammatory drug therapy. Cell numbers were assayed as discussed in Example 1.

As can be seen from Figure 7, monocyte numbers dropped to substantially zero during each CdA administration cycle. Monocyte numbers then returned to approximately the original, pre-treatment, number within about ten days after infusion of CdA was stopped for each cycle.

EXAMPLE 8: Synthesis of 2-chloro-9-1'-beta -2'-deoxy-2'-fluoro-D-arabinofuranosyl adenine

1',3'-Di-O-acetyl-5'-O-benzoyl-2'-deoxy-2'-fluoro-beta-D-arabinose (4.7 gm., 13.8 mmol) is added to 1 M HBr/CH<sub>2</sub>Cl<sub>2</sub> at zero degrees C then maintained at 5 degrees C for 24 hours. The solvent is removed by rotoevaporation under negative pressure, and the dry product is dissolved in dry toluene. The product is dried by rotoevaporation under reduced pressure to yield 3'-O-acetyl-5'-O-benzoyl-2'-deoxy-2-fluoro-D-arabinofuranosyl bromide (ABFA).

ABFA is dissolved in 200 ml of dichloroethane. 2,6-Dichloropurine (2.61 gm, 13.8 mmol) is added to the ABFA solution and the mixture is heated under reflux at 100 degrees C for 16 hours. The solution is then filtered

and rotoevaporated to dryness under reduced pressure. The dried powder is dissolved in  $\text{CHCl}_3$  and purified by flash chromatography (200 gm silica gel, 230-400 mesh, elution with 2:1 cyclohexane-ethyl acetate) to yield 2,6-dichloro-9,1'-(3'-O-acetyl-5'-O-benzoyl-2'-deoxy-2'-fluoro-beta-D-arabinofuranosyl)-9-purine. This 2'-fluoro-arabinofuranosyl derivative of 2,6-dichloropurine is reacted with methanolic ammonia to produce a 2-chloro-9-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine.

Thereafter, the solvent is removed by rotoevaporation and the resulting residue is washed by stirring in cold (4 degrees C) water (20 ml). The product is collected by filtration. The major product is purified by flash silica column chromatography (EtOAc:Methyl alcohol at 20:1), concentrated to a white powder by rotoevaporation and identified by NMR as 2-chloro-9,1'-beta-2'-deoxy-2'fluoro-D-arabinofuranosyladenine.

A similar result is obtained by reaction of ABFA (above) with 2,6-dichloropurine in sodium hydride/acetonitrile as discussed in Wright et al. (1987) J. Org. Chem., 52:4617-4618, followed by reaction with methanolic ammonia, as discussed above.

EXAMPLE 9: Synthesis of 2'-deoxyadenosine-1-N-oxide

2'-Deoxyadenosine (30 micromoles) in 5 ml of  $\text{NH}_4\text{HCO}_3$  at pH 5.5 was admixed with 120 mmoles of the magnesium salt of monoperphthalic acid at a temperature of zero degrees C with continuous mixing.

After a time period of 12 hours, the mixture was lyophilized, dissolved in 2 ml of water and applied to the top of a 20 ml chromatography column of Dowex AGIX-8 (formate form).

The 1-N-oxide was eluted with 0.1 M  $\text{NH}_4\text{HCO}_3$ .

EXAMPLE 10: Compressed Tablet

|    |                              |                          |
|----|------------------------------|--------------------------|
| 5  | <u>Ingredient</u>            | <u>Amount, mg/Tablet</u> |
|    | 2-chloro-9,1'-beta-          |                          |
|    | 2'-deoxy-2'-fluoro-D-        |                          |
|    | arabinofuranosyladenine      | 1                        |
|    | dibasic calcium phosphate NF | q.s.                     |
| 10 | starch USP                   | 40                       |
|    | modified starch              | 10                       |
|    | magnesium stearate USP       | 1-5                      |

EXAMPLE 11: Hard Shell Capsule

|    |                         |                           |
|----|-------------------------|---------------------------|
| 15 | <u>Ingredient</u>       | <u>Amount, mg/Capsule</u> |
|    | 2-methyl-9,1'-beta-     |                           |
|    | 2'-deoxy-2'-fluoro-D-   |                           |
|    | arabinofuranosyladenine | 1                         |
| 20 | lactose, spray dried    | q.s.                      |
|    | magnesium stearate      | 1-10                      |

EXAMPLE 12: Oral Liquid (Syrup)

|    |                         |                           |
|----|-------------------------|---------------------------|
| 25 | <u>Ingredient</u>       | <u>Amount, % wt./vol.</u> |
|    | 2-hydroxy-9,1'-beta-    |                           |
|    | 2'-deoxy-2'-fluoro-D-   |                           |
|    | arabinofuranosyladenine | 0.5                       |
|    | liquid sugar            | 75.0                      |
| 30 | methyl paraben USP      | 0.18                      |
|    | propyl paraben USP      | 0.02                      |
|    | flavoring agent         | q.s.                      |
|    | purified water, q.s. ad | 100.0                     |

EXAMPLE 13: I.V. Injectable Solution Concentrate

|   | <u>Ingredient</u>                                   | <u>Amount, % wt./vol.</u> |
|---|---|---------------------------|
| 5 | 2-chloro-9,1'-beta-<br>2'-deoxy-adenosine-1-N-oxide | 0.1                       |
|   | benzyl alcohol NF                                   | 0.9                       |
|   | purified water                                      | 100.0                     |

10 EXAMPLE 14: Enteric Coated Adenine Derivative

Table 1 lists the components of a drug composition of the present invention (Composition A) and an enteric coating composition (Compound B).

15

Table 1

## Composition A

|    | <u>Ingredient</u>                    | <u>Weight %</u> |
|----|--------------------------------------|-----------------|
| 20 | 2-Chloro-9,1'-beta-2'-deoxyadenosine | 67.0            |
|    | Polyvinylpyrrolidone                 | 1.3             |
|    | Modified Starch                      | 5.0             |
|    | Sodium Bicarbonate (anhydrous)       | 20.0            |
|    | Citric Acid                          | <u>6.7</u>      |
| 25 |                                      | 100.0           |

## Composition B

|    | <u>Ingredient</u>           | <u>Weight %</u> |
|----|-----------------------------|-----------------|
|    | Chloroform                  | 66.4            |
|    | Methanol (anhydrous)        | 15.4            |
| 30 | Cellulose Acetate Phthalate | 7.2             |
|    | Talc # 127 U.S.P.           | 7.3             |
|    | FD & C # 5 Yellow           | 1.0             |
|    | Diethyl Phthalate           | <u>2.7</u>      |
|    |                             | 100.0           |

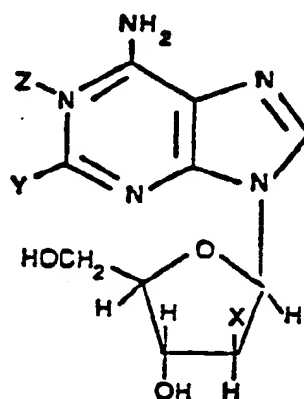
The ingredients listed for Composition A are mixed, together with the slow addition of anhydrous isopropyl alcohol (700 ml. per kg of Composition A) for about 9 to 15 minutes. The resulting blend is then segmented into tablets by extrusion. These segmented particles are dried in an oven at 35 degrees C for about 40 to about 48 hours. The dried granules are sized through a 14 mesh screen. Those segments that pass through the screen are compressed in a tablet machine to produce tablets about 4.8 mm in diameter and about 4 mm thick.

The dried tablets are then coated with the pH sensitive enteric coating composition (Composition B) in a pan employing about 0.45 liters of Composition B per kilogram of tablets to give a uniform coating weighing about 5.5% by weight of the final tablet. The wet coated tablets are then dried.

The foregoing description and the examples are intended as illustrative and are not to be taken as limiting. Still other variations within the spirit and scope of this invention are possible and will readily present themselves to those skilled in the art.

## WHAT IS CLAIMED IS:

1. A substituted adenine derivative represented by the formula:



wherein Z is O<sup>-</sup> or absent,  
Y is hydrogen or a substituent containing one to about 20 atoms that is free from net ionic charge at physiological pH values, provides a soluble adenine derivative, and whose presence on the adenine moiety inhibits deamination of the adenine derivative by adenosine deaminase, and

X is hydrogen or fluoro, with the provisos that:

i) when Z is absent, X is fluoro; and

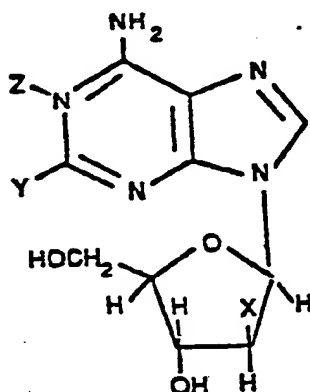
ii) Y is hydrogen only when Z is present and X is fluoro.

2. The adenine derivative of claim 1 wherein Y is a radical selected from the group consisting of halogen, lower alkyl, hydroxyl, lower alkylthio and lower alkanoylamido radicals.

3. The adenine derivative of claim 2 wherein Y is a halogen selected from the group consisting of fluorine, chlorine and bromine.

4. The adenine derivative of claim 1 wherein Y is chlorine, X is hydrogen and Z is  $O^-$ .

5. A pharmaceutical composition comprising a substituted adenine derivative as an active ingredient and a physiologically tolerable carrier, wherein said substituted adenine derivative is present in a therapeutically effective dose and has a structure that corresponds to the formula:

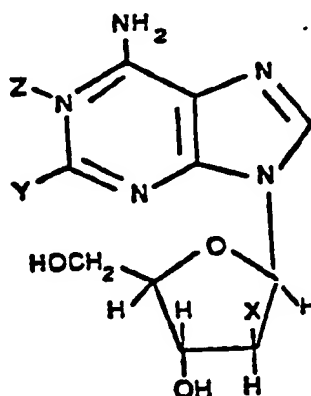


where Z is  $O^-$  or absent; Y is hydrogen or a substituent containing one to about 20 atoms that is free from net ionic charge at physiological pH values, produces a soluble adenine derivative, and whose presence on the adenine moiety inhibits deamination of said adenine derivative by adenosine deaminase; and X is hydrogen or fluoro, with the provisos that (i) when Z is absent, X is fluoro, and (ii) Y is hydrogen only when Z is present and X is fluoro.

6. A method of treating a monocyte-mediated disease state in a mammal comprising:



administering a composition containing a therapeutically effective dose of a substituted adenine derivative as an active ingredient dissolved or dispersed in a physiologically tolerable carrier to said mammal, said substituted adenine derivative being administered in an amount sufficient to decrease the level of monocytes in the blood of said mammal by at least about 50 percent during the course of said treatment and having a structure represented by the formula:



where Z is O<sup>-</sup> or absent; Y is hydrogen or a substituent containing one to about 20 atoms that is free from net ionic charge at physiological pH values, produces a soluble adenine derivative, and whose presence on the adenine moiety inhibits deamination of said adenine derivative by adenosine deaminase; and

X is hydrogen or fluoro, with the proviso that when Y is hydrogen only when Z is present.

7. The method of claim 6 wherein said monocyte-mediated disease state is an infective disorder in which microorganisms reside in infected monocytes.

8. The method of claim 6 wherein said Y-substituent is a radical selected from the group consisting of halogen, lower alkyl, hydroxyl, lower alkylthio and lower alkanoylamido radicals.

5 9. The method of claim 8 wherein Y is a halogen selected from the group consisting of fluorine, chlorine and bromine.

10 10. The method of claim 9 wherein X is fluorine.

11 11. The method of claim 6 wherein Y is chlorine, X is hydrogen, and Z is O<sup>-</sup>.

12 12. The method of claim 6 wherein said administration provides said adenine derivative in the plasma of said host mammal in amount of about 0.04 to  
15 about 0.20 milligrams per kilogram of host mammal body weight per day.

13. The method of claim 10 wherein said adenine derivative is administered perorally.

14 14. The method of claim 7, further comprising the administration of a therapeutically effective amount of an antimicrobial agent.

15 15. The method of claim 14 wherein said antimicrobial agent is administered separately to said host mammal from the administration of said substituted adenine derivative.

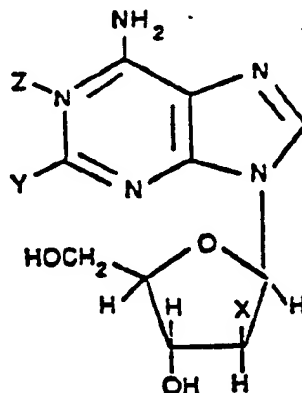
16. The method of claim 14 wherein said antimicrobial agent is an antiviral agent.

17. The method of claim 16 wherein said antiviral agent is azidothymidine.

18 18. The method of claim 14 wherein said antimicrobial agent is selected from the group consisting of nifurtimox, stibogluconate, amphotericin B, pentamidine isethionate, pyrimethamine, sulfonamide, chloroquine,

primaquine, mefloquine, trimethoprim-sulfamethoxazole, prednisone, etidronate sodium and sulfasalazine.

19. A method for the suppression of an immune response in a mammal comprising administering to said mammal a therapeutically effective dose of a substituted adenine derivative as an active ingredient dissolved or dispersed in a pharmacologically acceptable carrier, said adenine derivative having a structure represented by the formula:



wherein Z is O<sup>-</sup> or absent; Y is hydrogen or a substituent containing one to about 20 atoms that is free from net ionic charge at physiological pH values, produces a soluble adenine derivative, and whose presence on the adenine moiety inhibits deamination of said adenine derivative by adenosine deaminase, and X is hydrogen or fluoro with the proviso that Y is hydrogen only when Z is present, and pharmaceutically acceptable salts thereof.

20. The method of claim 19 wherein said adenine derivative is 2-chloro-2'-deoxy-2'-fluoro-9,1'-beta-arabinofuranosyladenine.

21. The method of claim 19 wherein said adenine derivative is administered in an amount sufficient to achieve a plasma concentration of about 1 nM to about 100 nM in said mammal.

22. The method of claim 19 comprising administering 2-chloro-2'-deoxy-2'-fluoro- 9,1'-beta-adenosine-1-oxide as an active ingredient to a mammalian host.

23. The method of claim 22 wherein said 2-chloro-2'-deoxy-2'-fluoro-9,1'-beta-adenosine-1-N-oxide is administered in an amount sufficient to supply the blood stream of said host at a dose rate of about 0.04 to about 0.20 milligrams per kilogram of host body weight per day.

24. The method of claim 19 wherein said mammal is a human.

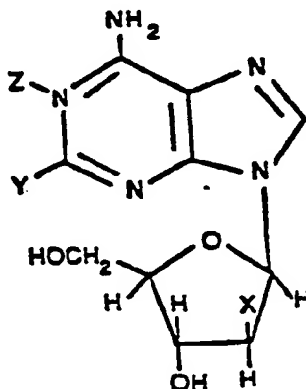
25. The method of claim 22 wherein said 2-chloro-2'-deoxy-2'-fluoro-9,1'-beta-adenosine-1-oxide is dissolved or dispersed in a physiologically tolerable diluent.

26. The method of claim 19 wherein said mammal has an autoimmune disease.

27. The method of claim 26 wherein said autoimmune disease is rheumatoid arthritis.

28. A method of treatment for acquired immune deficiency syndrome in a human comprising:

a) administering to said human a pharmaceutical composition containing a therapeutically effective dose of a substituted-2'-deoxy-arabinofuranosyl-adenine derivative having a structure corresponding to the formula:



as an active ingredient dissolved or dispersed therein,  
where Z is O<sup>-</sup> or absent; Y is hydrogen or a  
substituent containing one to about 20 atoms that is free  
from net ionic charge at physiological pH values, produces  
5 a soluble adenine derivative, and whose presence on the  
adenine moiety prevents deamination of said adenine  
derivative by adenosine deaminase; and X is hydrogen or  
fluoro, with the proviso that Y is hydrogen only when Z is  
present.

10 29. The method according to claim 28, further  
comprising the administration of a therapeutically  
effective dose of an antiviral agent.

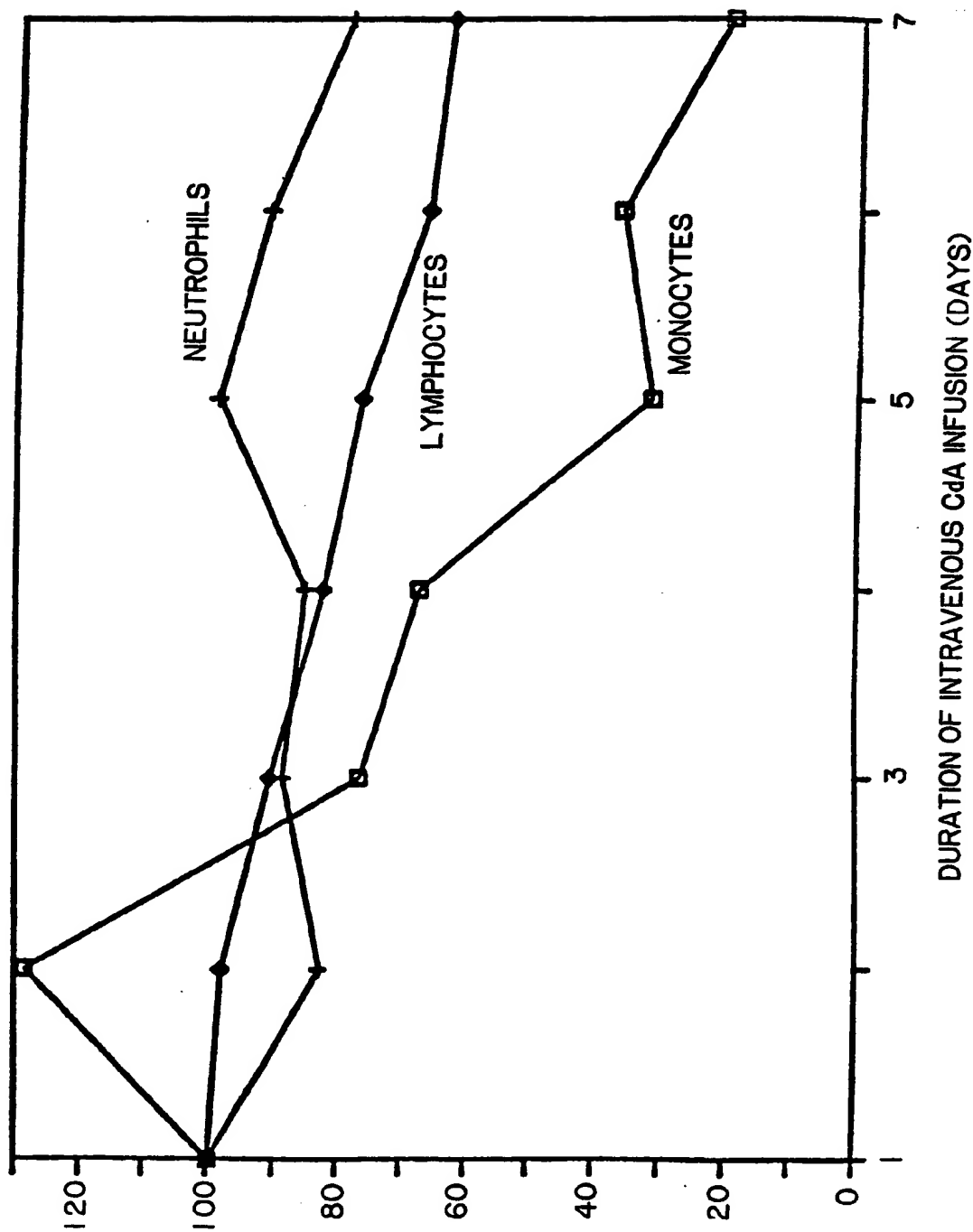
15 30. The method of claim 29 wherein said  
antiviral agent is administered separately to said human  
from the administration of said substituted-2'-  
deoxyarabinofuranosyladenine derivative.

20 31. The method according to claim 29 wherein  
said antiviral agent is selected from the group consisting  
of azidothymidine, 2'3'-dideoxycytidine, acyclovir and  
interferon.

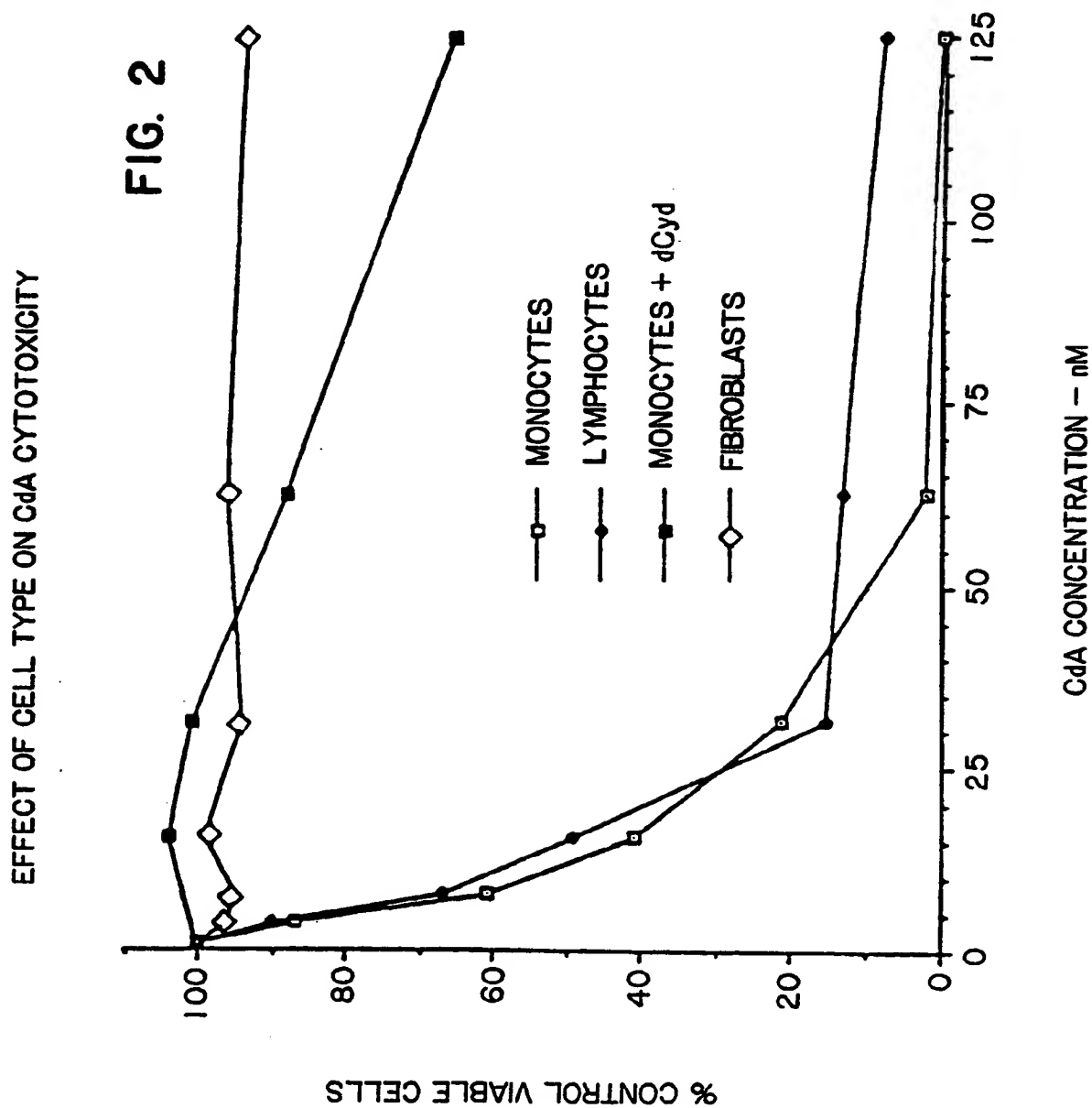
25 32. The method according to claim 31 wherein  
said substituted-2'-deoxy-arabinofuranosyladenine  
derivative is 2-chloro-2'-deoxyadenosine administered at  
about 0.04 to about 0.20 mg per kg body weight per day  
together with a therapeutically effective amount of  
azidothymidine.

FIG. 1

HEMATOLOGIC EFFECTS OF C<sub>14</sub>A



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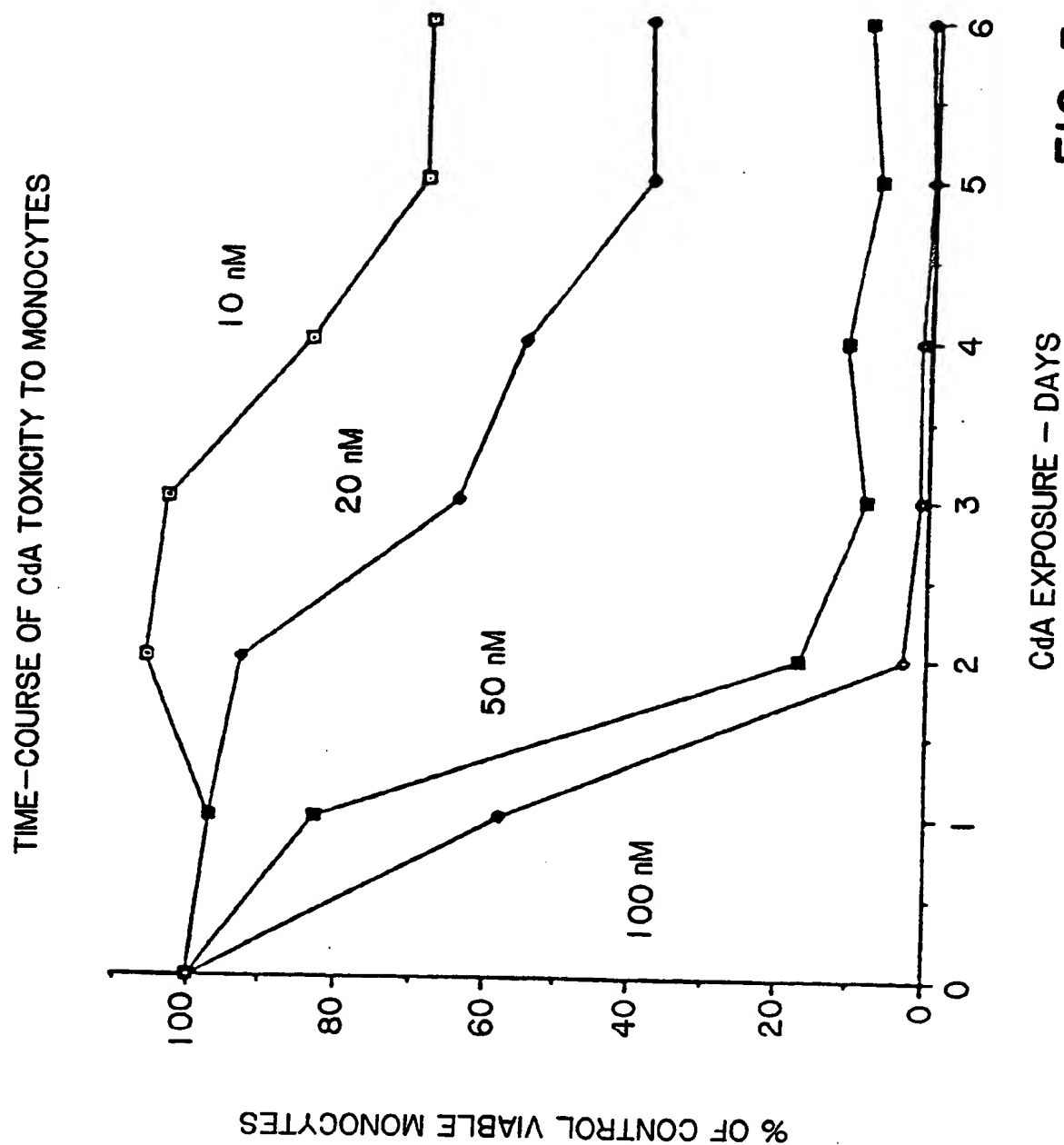


FIG. 3



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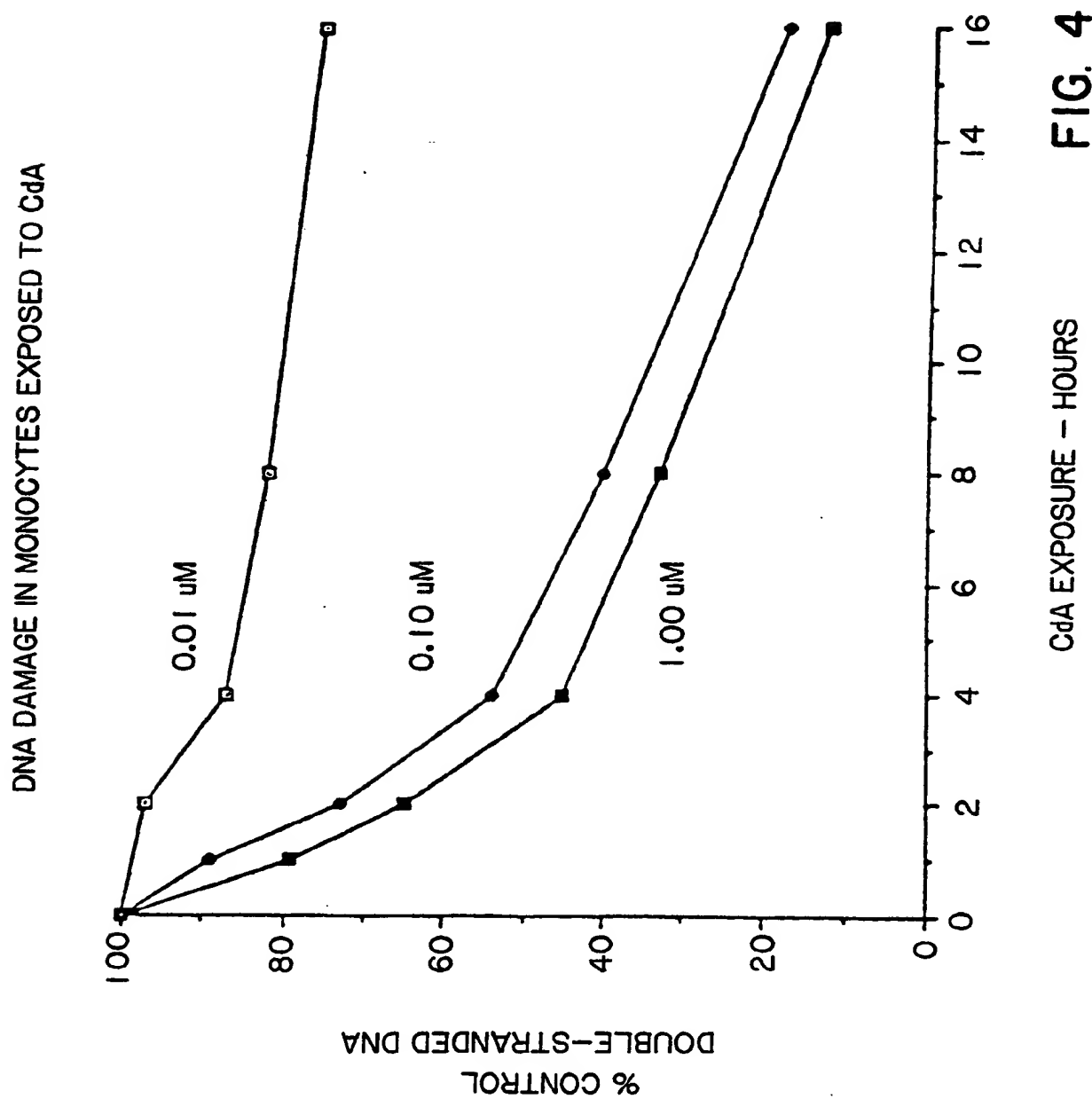
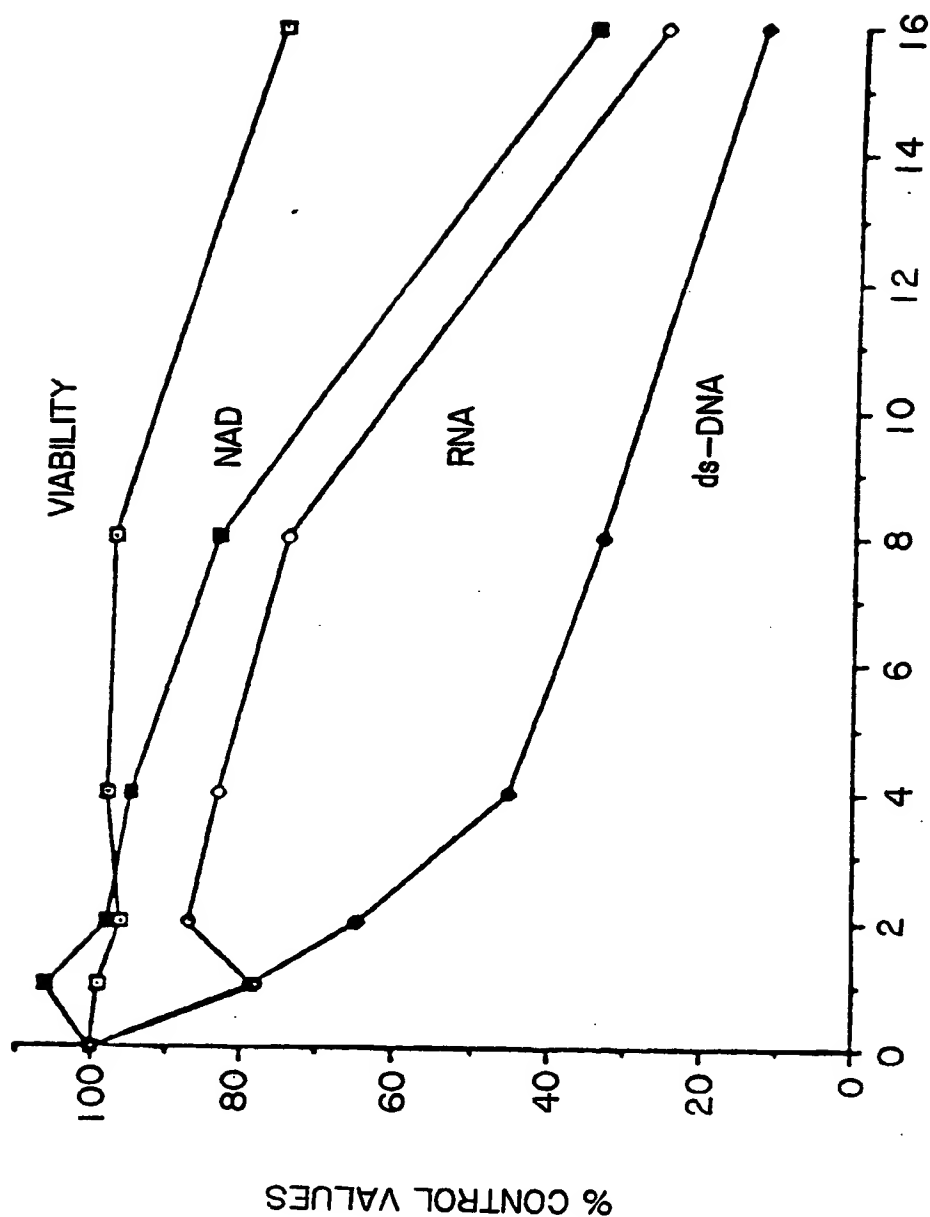


FIG. 4

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BIOCHEMICAL EFFECTS OF CdA ON MONOCYTES



CdA EXPOSURE - HOURS

FIG. 5

SUBSTITUTE SHEET

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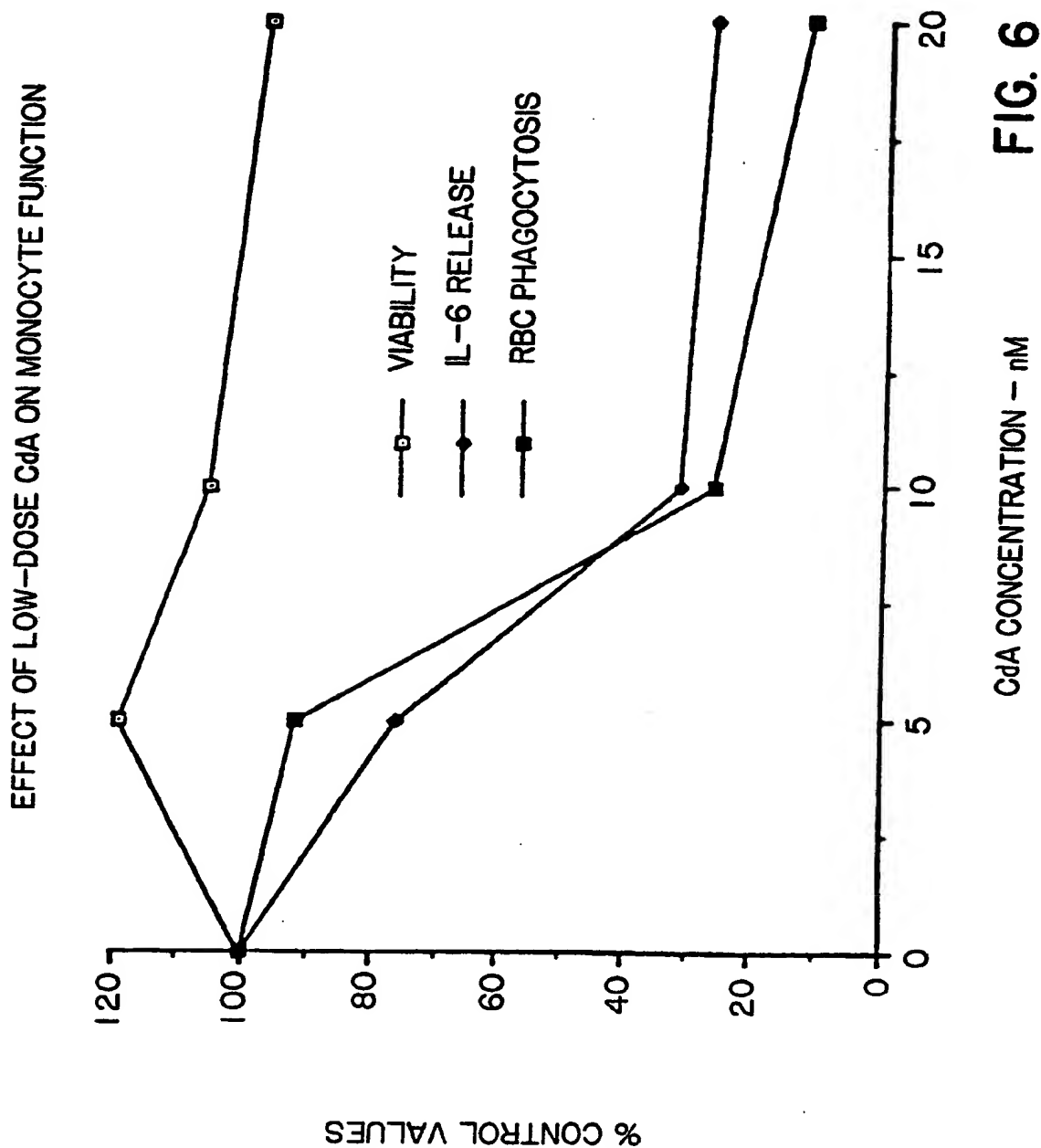


FIG. 6

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BLOOD CELL COUNTS DURING C<sub>4</sub>A THERAPY  
PATIENT 1 - RHEUMATOID ARTHRITIS

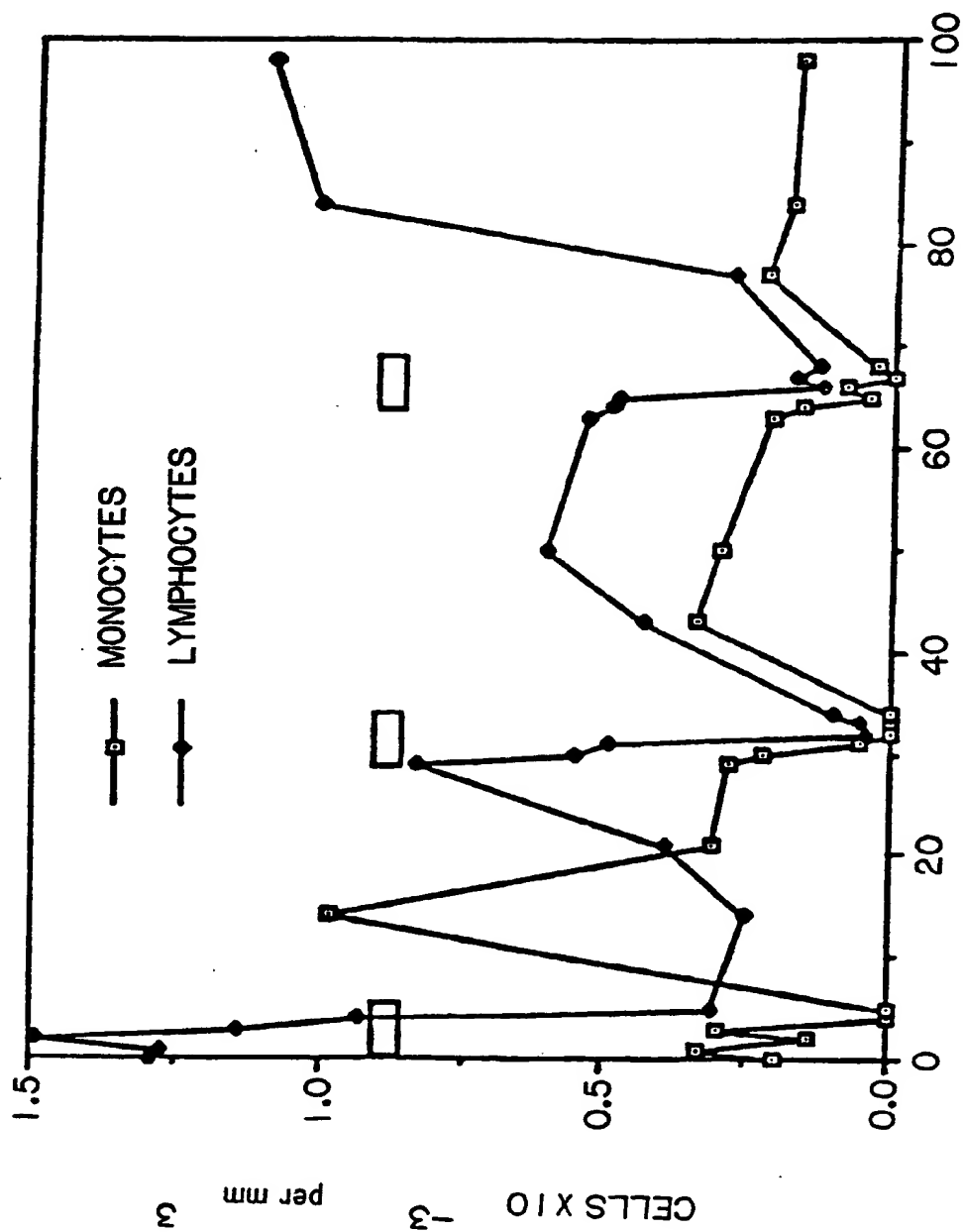


FIG. 7

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/01088

|  |  |   |                       |                        |        |                                |
|--|--|---|-----------------------|------------------------|--------|--------------------------------|
| <b>I. CLASSIFICATION &amp; SUBJECT MATTER</b> (In several classification symbols apply, in the case all) :<br>According to International Patent Classification (IPC) or to both National Classification and IPC<br>IPC(4th Ed.): C 07H 19/19; A61K 31/70<br>US Cl.: 514/45; 514/46; 536/24; 536/26   |  |   |                       |                        |        |                                |
| <b>II. FIELDS SEARCHED</b><br><div style="text-align: center; font-size: small;">Minimum Documentation Searched *</div> <table style="width: 100%; border: none;"> <tr> <td style="width: 30%; border: none;">Classification System  </td> <td style="border: none;">Classification Symbols</td> </tr> <tr> <td style="border: none;">US Cl.</td> <td style="border: none;">514/45; 514/46; 536/24; 536/26</td> </tr> </table> <div style="text-align: center; font-size: x-small; margin-top: 10px;">Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched *</div>  |  |   | Classification System | Classification Symbols | US Cl. | 514/45; 514/46; 536/24; 536/26 |
| Classification System  | Classification Symbols   |   |                       |                        |        |                                |
| US Cl.   | 514/45; 514/46; 536/24; 536/26   |   |                       |                        |        |                                |
| <b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> <sup>14</sup>  |  |   |                       |                        |        |                                |
| Category *   | Citation of Document, <sup>15</sup> with indication, where appropriate, of the relevant passages <sup>17</sup> | Relevant to Claim No. <sup>16</sup>                   |                       |                        |        |                                |
| Y  | US, A, 4,526,988, HERTEL, Published 02 July 1985, see columns 1-16.  | 1-32  |                       |                        |        |                                |
| Y  | US, A, 4,692,434, HERTEL, Published 08 September 1987, see columns 1-20.                                       | 1-32  |                       |                        |        |                                |
| Y  | US, A, 3,472,838, HANESSIAN, Published 14 October 1969, see columns 1-6.                                       | 1-32  |                       |                        |        |                                |
| Y  | US, A, 3,666,856, ELION ET AL., Published 30 May 1972, see columns 1-4.  | 1-32  |                       |                        |        |                                |
| Y  | US, A, 3,956,277, ELION ET AL., Published 11 May 1976, see columns 1-10.                                       | 1-32  |                       |                        |        |                                |
| Y  | US, A, 4,081,534, ELION ET AL., Published 28 March 1978, see columns 1-6                                       | 1-32  |                       |                        |        |                                |
| <div style="display: flex; justify-content: space-between; font-size: x-small;"> <div style="width: 45%;"> <p>* Special categories of cited documents: <sup>15</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div> |  |   |                       |                        |        |                                |
| <b>IV. CERTIFICATION</b>   |  |   |                       |                        |        |                                |
| Date of the Actual Completion of the International Search *  |  | Date of Mailing of this International Search Report * |                       |                        |        |                                |
| 12 June 1989   |  | 24 JUL 1989   |                       |                        |        |                                |
| International Searching Authority *  |  | Signature of Authorized Officer <sup>18</sup>         |                       |                        |        |                                |
| ISA/US   |  | L. Eric Crane   |                       |                        |        |                                |

| FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET  |  |      |
|--|--|------|
| Y  | GB, B, 1,386,585, THE WELLCOME FOUNDATION, LIMITED, Published 12 March 1975, see all columns, pages 1-7. | 1-32 |
| P, Y   | E P, A, 0,287,313, THE UNITED STATES OF AMERICA, Published 19 October 1988, see columns 1-9.             | 1-32 |
| <b>V. <input type="checkbox"/> OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>10</sup></b><br><p>This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:</p> <p>1. <input type="checkbox"/> Claim numbers ..... because they relate to subject matter <sup>11</sup> not required to be searched by this Authority, namely:</p> <p>2. <input type="checkbox"/> Claim numbers ..... because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out <sup>12</sup>, specifically:</p>  |  |      |
| <b>VI. <input type="checkbox"/> OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>13</sup></b><br><p>This International Searching Authority found multiple inventions in this international application as follows:</p> <p>1. <input type="checkbox"/> As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.</p> <p>2. <input type="checkbox"/> As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically: "claims:</p> <p>3. <input type="checkbox"/> No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:</p> <p>4. <input type="checkbox"/> As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.</p> <p>Remark on Protest</p> <p><input type="checkbox"/> The additional search fees were accompanied by applicant's protest.</p> <p><input type="checkbox"/> No protest accompanied the payment of additional search fees.</p> |  |      |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE COND SHEET) |  |                         |
|--|--|-------------------------|
| Category *   | Citation of Document, with indication, where appropriate, of the relevant passages *                                       | Relevant to Claim No. * |
| Y  | US, A, 3,539,550, GREENBERG ET AL.,<br>Published 10 November 1970, see columns<br>1-12.                                    | 1-32                    |
| Y  | ORGANIC CHEMISTRY OF NUCLEIC ACIDS,<br>Part B, Kochetkov et al., 1972, Plenum Press,<br>New York, N.Y., p. 335, lines 1-9. | 1-32                    |